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CONTROL OF PHYTOPHTHORA HEART ROT OF PINEAPPLE PLANTS[1]

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INTRODUCTION

This paper presents the results of 2 years of studies designed to give an effective and economical control of heart rot of the pineapple plant, which in Hawaii is caused by *Phytophthora cinnamomi* Rands, *Ph. palmivora* Butl. (*Ph. meadii* McRae), and *Ph. parasitica* Dastur. The last-named species is interpreted to include *Ph. melongenae* Sawada. Heart rot caused by each of these 3 species is so closely similar that, for present purposes, no distinctions are necessary. Details of the local and wide-spread geographic distribution of the pathogens, their physiology, morphology, and taxonomy, in addition to information concerning the epidemiology of the disease, are presented in a separate paper (12). A prior report of heart rot of the pineapple plant in Hawaii, not including control measures, has been presented by Sideris and Paxton (13).

The Disease and Its Occurrence

Heart rot, as suggested by the name, is a rot of the heart of the plant, which destroys the younger portions of the stem and the bases of the central leaves. The outermost leaves characteristically escape invasion, as do the older parts of the stem. In very young plants, however, all of these regions may be involved. Heart rot also is known as top rot (15).

In the field heart rot of young plants is recognized by a series of color changes of the leaves, a lack of growth of the plant, and a ready removal of the entire heart of the plant when the central leaves are lightly pulled. The leaves of a diseased plant first become reddish, but soon change to a pallid yellow. Before the color change is apparent, the bases of affected leaves display a transparent water-soaked area. After the change in color is noticeable the leaf base will be found to display a malodorous, yellow-white rotted area, bordered by a very distinct and characteristic brown margin (Fig. 1, B). This area ordinarily extends into the stem tissue below, but is limited quite sharply by the green parts of the leaf above. The invaded stem tissue is of a cheesy consistency. It charac-

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teristically is similar to the leaf lesions in odor, color, and possession of the well-defined brown margin. Plants in advanced stages of heart rot may display a falling apart of the leaves. The older basal parts of the stems of larger plants usually are not rotted by *Phytophthora* spp. and may remain sound. Secondary organisms, both fungi and bacteria, rapidly follow the casusal organism, decomposing the invaded tissues changing the odor from that characteristic of the disease.

The phase of heart rot described above agrees with that mentioned in the scant literature regarding the disease (1, 2, 5, 7, 8, 11, 13, 15). It is this phase with which the present study is concerned. Another phase of

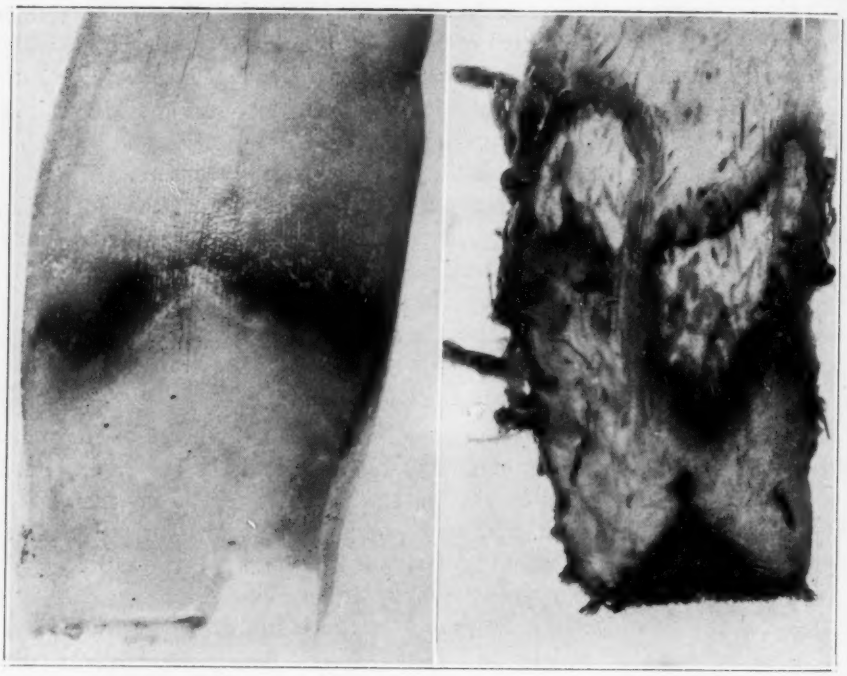

FIG. 1. Pineapple heart rot caused by *Phytophthora cinnamomi*. Note the rotted white areas bordered by the characteristic brown margin. A. Stem lesion. B. Leaf lesion.

the disease, not reported by others, affects older, more mature plants. Plants old enough to flower and even those which have already borne fruit may succumb (Fig. 2). The lesions displayed by the stem and leaves of older plants are similar in all respects to those described above for younger plants. The central leaves of such diseased plants, being separated from their vascular supply, lose their turgidity, collapse, and give the plant a prostrate appearance. The older, more fibrous parts of the stem are rarely

invaded. They may give rise to new growth from previously dormant axillary buds either above or below the soil. This new growth may remain healthy, reestablishing the plant, or may succumb. Suckers from healthy plants may be killed by heart rot without any apparent effect on the parent plant. Stem lesions in older plants do not always involve the leaves. In such cases the plant becomes stunted and the narrower, stiffer leaves show an abnormal reddening.

The losses from heart rot of young plants occur intensively chiefly within very limited regions of large fields. In the affected areas, ordinarily

FIG. 2. Near view of a pineapple plant failing from heart rot after having borne fruit, compared with an adjacent healthy plant of the same age. The shoot in the foreground as well as the slips all show the results of infection.

two acres or less in extent, 50 to 75 per cent of the plants may be destroyed. Losses of more than 15 per cent of all the plants in a 100-acre field have been observed, but such wide-spread destruction is characteristic only under the most adverse conditions. Poor physical condition of the soil, inadequate drainage, and flooding intensify the losses. Figure 3 shows a characteristic heart-rot area, where large vacant spaces have resulted from the removal of diseased plants.

Within the territory of Hawaii the disease has been observed on the islands of Oahu, Molokai, Lanai, Kauai and Maui. It has been found in soils ranging in pH from 3.5 to above 7.0. It occurs in areas with an an-

nual rainfall in excess of 100 inches and, as well, in areas averaging less than 20 inches a year. In regions where the disease reaches its maximum severity, light showers characterize the usual day, and days with no precipitation are infrequent. At the sites of experiments 7 and 8, reported below, 30 inches of rain fell within a month and 15.5 inches were recorded for a single day.

Former Attempts at Control

Sideris and Paxton (13) recommended improvement of soil drainage and aeration for limiting losses from *Phytophthora* spp. in Hawaii but presented no experimental evidence of the efficiency of such measures. In many areas such improvement alone is ineffective as a control.

Johnson (6) states that Ashby found spraying with hot Bordeaux beneficial in Jamaica and that Siggers in Costa Rica used a lime sulphur dust.

FIG. 3. Molokai control experiment after 5 months. The plots are, left to right: Check, no treatment; Bordeaux dip 1–0.7–3; check; Niagara colloidal sulphur dust.

Methods of application, concentrations used, or detailed results of control effected are not presented.

In 1930 M. B. Linford and G. E. Paxton, of this Station, undertook preliminary studies of control by fungicides. Their unpublished notes suggest that, to be most effective, fungicides should be applied to the planting material before setting it in the field, or that the newly set plants should be immediately treated with quantities of liquid fungicides sufficient to wet the soil around the butt of each plant. They used representative copper and mercury fungicides, aluminum sulphate, sulphuric acid, and a colloidal sulphur in limited field tests and concluded that Bordeaux mixture, Burgundy mixture, Semesan, aluminum sulphate, and copper carbonate all showed some promise.

Requirements of a Successful Control Practice

A control measure, to be successful, must take into account a number of separate factors: it must protect the susceptible tissues both above and below ground and be a single application, if possible. It must be of a lasting nature to withstand heavy rains and possible flooding and must be adaptable to unpredictable major or minor occurrences of disease of local or widespread distribution.

The causal organisms all survive for long periods of time in the soil, whence they enter the host through wounds mostly below the surface of the soil. In rare cases wounds near the apex of the stem may serve as avenues of entrance. Evidence of penetration through sound tissues is yet inconclusive, although experimental studies suggest that *Phytophthora cinnamomi* may enter directly under certain conditions.

Standard planting practices in the Hawaiian Islands involve the use of 10,000 to 20,000 pineapple plants per acre. Only a small percentage of these, distributed over a large area, may become diseased, but the total numbers of plants treated in a single field may be many thousands. Because of the distances involved repeated treatments of any nature would involve high costs for labor.

The magnitude of losses from heart rot is dependent upon environmental factors, which may vary considerably within a given field from year to year; upon the age, vigor, and type of planting materials; and upon distribution, abundance, and species of the parasite. The varied types of information requisite to a prediction of losses in any locality are not now available.

EXPERIMENTAL STUDIES

Studies started by the writer in the fall of 1930 were designed to determine the concentrations of representative fungicides that could be placed on the tender tissues of planting materials and young growing plants without causing undue injury and to test under field conditions the controlling effect of various of the most desirable preparations.

Experiments to determine fungicides desirable for control tests in the field were carried on first in the laboratory and later in outdoor plots. Only the field tests will be reported.

To test plant tolerance to various fungicides, young, vigorously growing plants were treated in blocks of 6 to 10 each. Two grams of dust were sifted into the hearts and onto the leaves, or 250 cc. of freshly prepared liquid was poured into the hearts of each plant treated.

Table 1 presents for each fungicide only the highest concentration tested and tolerated, or the various significant ratios of ingredients that did not injure the test plants. Fungicides causing serious injury at the lowest concentrations used are not included in the table. The Bordeaux

TABLE 1.—*Fungicides tested and tolerated by young growing pineapple plants*

Fungicide	Concentration	Fungicide	Concentration
	Dusts		
Niagara colloidal sulphur	2 gm. per plant	Bayer Special II–6	2 gm. per plant
Lime sulphur	2 gm. per plant	Fungi Bordeaux	2 gm. per plant
Sublimed sulphur	2 gm. per plant	Copper carbonate	2 gm. per plant
Ferrox sulphur	2 gm. per plant		
	Liquid in Hearts		
Permek sulphur	1–2.5	Bordeaux	1–0.35–1
Ialine "	1–5	"	1–0.7–1
Nickel "	1–1.2	"	1–1.05–1
Lime "	1–11	"	1–0.7–1.5
" " + aluminum sulphate	1–0.65–15	"	1–0.7–2
Calogren + Kayso (20 g./gal.)	1–4.5	"	1–1.05–2
Copper carbonate	1–7	"	1–0.7–2.5
" " + sal soda	1–0.75–11	"	1–1.05–2.5
" " + ammonium carbonate	1–2–11	" + resin sticker	1–0.35–14
Copper carbonate + ammonium carbonate	1–3–15	" + " "	1–0.7–14
Copper carbonate + hydrated lime	1–2–11	" + iron sticker	1–0.7–3
Copper carbonate + hydrated lime + Kayso 20 g./gal.	1–1–15	" + Goulac sticker	1–0.7–2.5
Pyrox	1–2.5	" + Kayso sticker	1–0.7–2.5

mixtures used were made with fresh hydrated Waianae lime, a local lime produced at Waianae, Oahu, T. H.[2]

[2] An analysis of the lime used in the Bordeaux mixtures of tests described below is as follows:

Analysis	*Per cent*
Silica (SiO_2)	1.03
Iron and alumina (Fe_2O_3 and Al_2O_3)	1.46
Calcium oxide (CaO)	72.67
Magnesia (MgO)	1.53
Sulfates (SO_3)	0.15
Carbon dioxide (CO_2)	1.10
Moisture (H_2O)	0.69
Loss on ignition (Less CO_2)	22.06
Available CaO (Sugar soluble)	68.2
Fineness:	
Residue on a No. 30 sieve	.0098
Residue on a No. 200 sieve	.78

In all references to the Bordeaux mixtures used, the lime content will be expressed as the available calcium oxide equivalent of the hydrated lime; *i.e.*, a factor of 0.7 will be used.

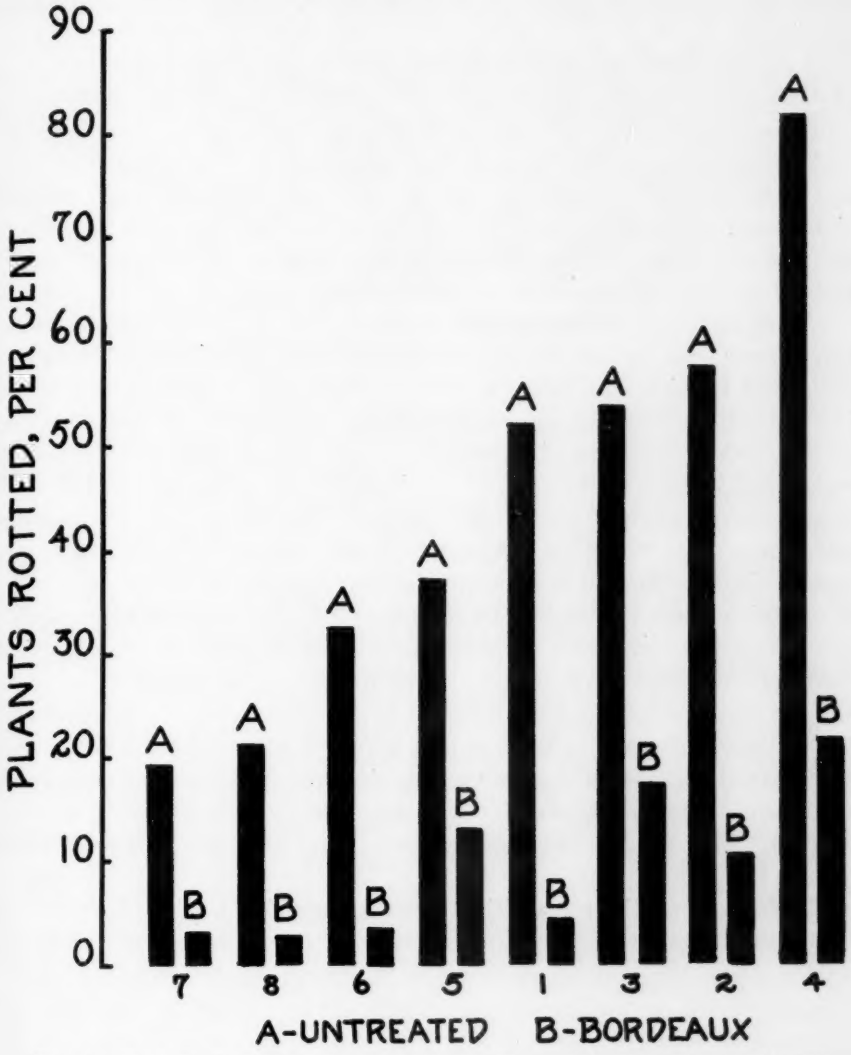

FIG. 4. Control of pineapple heart rot by Bordeaux 1–0.7–3 used as a dip. Numbers below the pairs of columns refer to the experiments discussed in the text. Experiments were conducted on 3 different islands.

Field Control Studies

During the planting seasons of 1930–1931, and 1931–1932, a number of field tests of half-acre or acre size were installed on the islands of Oahu,

Lanai, Molokai and Kauai. In a number of these experiments disease did not develop significantly in the check plots. In other instances complicating factors completely invalidated the data. Only those tests are reported where the effect of fungicides on the incidence of disease could be evaluated accurately.

Experiment 1: Factors of coverage, adherence, ease of preparation, lack of toxic effects to the host, and cost were considered in the selection of representative fungicides for the first field control test.

The site selected was characterized by repeated losses from heart rot, and by stunting of plants from root failure, both caused by *Phytophthora cinnamomi.* Drainage was poor, the surface soil shallow, and the subsoil compact and gummy. Planting material was large slips, except that 10 plots of crowns were also used as indicated below.

All slips except the checks were either dipped or dusted prior to planting. Dusts were applied by rotating the planting material in a barrel into which the fungicidal dusts were blown. The liquids were freshly mixed in the field; in them the appropriate plants were immersed momentarily.

There were 175 plots of 2 rows (one bed) each, averaging 33 feet long and containing about 70 plants. They included 9 replications of each of 15 treatments, 30 plots of untreated slips, and 10 plots of untreated crowns as controls. The 9 replications were in 3 series of 3 replications each, and these series were located in separate parts of the experimental area.

In this experiment, as in all others detailed below, counts of plants infected with heart rot were made periodically by lightly pulling the center leaves of each plant. All diseased plants were removed from the field when discovered. Six months from the time of planting, the active development of heart rot had ceased. Later counts revealed an occasional plant which was infected, but the numbers were insignificant. Heart rot developed to a significant degree in parts of the field occupied by series 1 and 2, but not series 3. This latter series was omitted, therefore, from computation of results.

Table 2 shows the composition of each fungicide used and, for series 1 and 2 only, the numbers of plants treated, the percentage of rot in the treated plots, the percentage of rot in adjacent checks, and that of the control effected by the treatments. Control was calculated from the percentage differences of disease in the treated plots and adjacent checks.

This test indicated that fungicides of many types may give a high degree of control if used in proper strength. Certain of the other preparations showed a high degree of control, but were toxic to the plants. Thus severe burn was apparent in plots treated with copper carbonate dust, dry lime sulphur dust, and copper carbonate aqueous suspension, but the injury was

not permanent in the lime sulphur plots. Burning by copper carbonate dust and copper carbonate liquid involved 81 and 55 per cent of the plants, respectively. The addition of hydrated lime to each reduced the burning of the dust to 37 per cent and of the liquid to 38 per cent. See treatments 8 and 12 in table 2.

TABLE 2.—*Results from heart-rot control experiment 1, showing the compositions of fungicides used, the total plants in each treatment, the percentage of rot in treated and check plots, and the percentage of control effected*

No.	Fungicide Composition	Plants treated	Rot in treated plots	Rot in checks	Control
Dips		*No.*	*Per cent*	*Per cent*	*Per cent*
1	Bordeaux 1–0.7–6	704	4.74	52.39	90.95
2	" 1–0.7–13.5 plus resin sticker	688	8.08	42.14	80.83
3	" 1–0.7–13.5 plus no sticker	678	6.71	39.86	83.17
4	" 1–0.35–13.5	695	11.90	39.76	70.08
5	" 1–0.35–13.5 plus resin sticker	675	8.01	37.80	78.81
6	Ialine sulphur 1–3	654	54.29	49.67	−9.3
7	Copper carbonate 1–12	709	2.65	48.59	94.55
8	" " plus hydrated lime 1–0.25–15	678	3.96	51.86	92.36
Dusts					
9	Bayer special II–6	702	15.06	32.36	53.47
10	Lime sulphur (dry)	640	3.75	51.0	92.65
11	Copper carbonate	692	0.0	38.57	100.00
12	" " plus hydrated lime 4–1	693	0.0	45.61	100.00
13	Ferrox sulphur	730	1.10	38.28	97.13
14	Fungi Bordeaux	699	0.0	34.58	100.00
15	Niagara colloidal sulphur, X compound	668	1.34	47.43	97.18

Experiment 2: The first control experiment of a significant size, using a concentration of Bordeaux higher than 1–0.7–6, was installed at Kipu, Molokai, January, 1931. In the area selected for this test more than 50 per cent of the plants had died of heart rot caused by *Phytophthora parasitica.* The remaining plants were removed. Plots, numbered 1 to 17 each, consisted of 4 adjacent rows of plants. The planting material used was well-cured crowns. From some of these the basal leaves were stripped before curing. Others were cured, treated, and planted without stripping.

Eight months after installation the percentage control effected by the

various treatments was calculated. Table 3 presents these data, also the number of replications of each treatment and their distribution. An analysis of the control data by Student's method for paired observations (10) showed that differences noted between Ferrox and Niagara sulphurs on the one hand, and between trimmed and untrimmed crowns on the other are not statistically significant. Differences between other treatments are highly significant. Figure 3 illustrates this experiment 5 months after planting.

TABLE 3.—*Control of heart rot by fungicides at Kipu, Molokai. The distribution of plots, the percentage of plants of each plot that rotted, and the percentage control of each fungicide is shown*

Nontreated check		Bordeaux Dip 1–0.7–3[a]				Niagara Colloidal Suphur		Ferrox Sulphur	
		Trimmed		Untrimmed					
Plot No.	Per cent of plants rotted	Row No.	Rotted plants	Row No.	Rotted plants	Row No.	Rotted plants	Row No.	Rotted plants
			Per cent		*Per cent*		*Per cent*		*Per cent*
1	51.22	2	7.14	6	12.28	3	69.12	5	48.48
4	50.59	6	27.27	11	5.71	8	54.26	9	65.41
7	66.67	11	10.00	15	15.49	12	64.34	14	60.40
10	62.77	15	12.16					17	70.99
13	63.70								
16	49.37								
Percentage control by fungicides:									
			75.57		84.42		−11.78		−8.19

[a] Trimmed and untrimmed crowns were planted in the same beds; trimmed ones were placed in the 2 left rows and untrimmed ones in the 2 right rows.

Experiment 3: During March, 1932, a half-acre test of 4 liquid fungicides and a dust was installed at Opaeula, Oahu. The liquids were applied by dipping, as in earlier tests, but the dust, Ferrox sulphur, was applied with a hand duster of the blower type. This experiment occupied an area where heart rot and root rot caused by *Phytophthora cinnamomi* had killed about 70 per cent of the first planting. Soil conditions were comparable to those in experiment 1 described above.

A modified Latin Square arrangement of plots was used, incorporating twice as many check plots as those of any single treatment. Each plot was 18 feet long and 2 beds wide. Approximately 600 plants were treated with each fungicide. The planting materials, having stood on the trimming grounds for many months, were of poor quality.

The pertinent data from experiment 3 are presented in tables 4 to 7. Table 4 shows the distribution of the plots of each treatment and gives the

TABLE 4.—*Showing the distribution of plots in experiment 3, and the percentage of disease in each plot after 6 months*[a]

A	C	E	G	F	B	D
30.2	50.0	67.3	21.4	14.1	15.2	26.4
G	B	F	A	D	E	C
46.3	16.3	19.7	50.0	27.6	52.9	59.8
E	F	A	D	C	G	B
56.3	28.1	43.0	29.3	47.6	25.5	21.1
B	D	G	F	A	C	E
25.3	52.4	52.2	18.4	91.3	57.7	64.3
F	A	B	C	E	D	G
44.7	78.6	14.5	57.9	68.1	24.7	37.5
D	G	C	E	B	A	F
30.3	18.9	56.0	58.0	18.9	44.9	18.8
C	E	D	B	G	F	A
64.9	54.0	5.6	14.8	25.0	20.7	29.6

[a] Treatment: A and C, nontreated checks; B, Bordeaux dip 1–0.7–3; D, Ferrox sulphur dust; E, Bayer Special II–6; F, copper carbonate with lime dip 2–1–15; G, Ansul wettable sulphur dip 1–6.

percentage of diseased plants in each plot after 6 months. From these data the control effected by the various treatments was calculated (Table 5).

TABLE 5.—*The composition of fungicides, the numbers of plants treated, the mean percentage of diseased plants in each treatment, and the control effected by the fungicides in experiment 3*

	Treatment	Plants treated	Mean per cent rot	Control effected
A & C	Nontreated check	1109	54.4	
B	Bordeaux dip 1–0.7–3	546	18.0	66.92
D	Ferrox sulphur dust	598	28.0	48.53
E	Bayer II–6 dip 1–3	600	60.1	−11.05
F	Copper carbonate plus hydrated lime 2–1–15	584	23.5	56.81
G	Ansul wettable sulphur 1–6	531	32.4	40.45

Fisher's method for the analysis of Latin Square experiments permits an evaluation of the significance of the experiment as a whole, as well as of the individual treatments. Table 6 is the analysis of variance of experiment 3 by Fisher's method (4).

TABLE 6.—*Analysis of variance of experiment 3*

Source of variance	Degrees of freedom	Sum of squares	Variance	$\frac{1}{2}$ log$_e$ variance
Rows	6	2506.3	417.7	3.0180
Columns	6	513.4	85.6	2.2239
Treatments	5[a]	12347.3	2469.5	3.9066
Remainder	31	3700.2	119.4	2.8917
Total	48	19067.2		

[a] The identical treatments A and C are considered as a single check in this analysis.

The variance due to treatments is very significant for the difference between the values $\frac{1}{2}$ log$_e$ variance of the treatments and remainder greatly exceeds the value .4164, which is the deviation that would be exceeded by chance in 5 per cent of random samples (See (3), table VI).

The significance of variance between the several treatments of experiment 3 determined by Fisher's method is given in table 7.

TABLE 7.—*Significance of variance between treatments of experiment 3*

	Treatments						Mean	Standard error
	Check (A + C)/2	B	D	E	F	G		
Per cent plants rotted[a]	380.8	126.1	196.3	420.9	164.5	226.8	270.8	26.77
Per cent of mean	140.6	46.6	72.5	155.4	60.8	83.8	100.0	10.67[b]

[a] If all plants in any treatment were rotted the value would be 700, the sum of the 7 replicates.

[b] To be significant the differences between pairs of treatments must exceed $2\sqrt{2 \times 10.67} = 30.17$.

The effectiveness of treatments judged by Student's method (14) forms the same series as that by Fisher's method.

Note, in table 4, the spotted distribution of disease shown by the spread of the percentage of rotted plants in the plots of check A from 29.6 to 91.3 per cent. Plots of check C show a range from 47.6 to 64.9 per cent only.

Despite this demonstrated heterogeneity no statistically significant differences occurred between series A and C. This fact seems to justify the distribution of plots that was used.

Using the information in tables 5 and 7 as a basis, the treatments of experiment 3 may be arranged in a series ranging from the most effective to the least effective: (1) Bordeaux dip 1–0.7–3, (2) copper carbonate and lime dip 2–1–15, (3) Ferrox sulphur dust, (4) Ansul wettable sulphur dip 1–6, and (5) Bayer Special II–6 dip 1–3. The last-named treatment was without significant effect on the occurrence of heart rot.

Experiment 4: The experiment discussed below was installed on the island of Lanai during September, 1931. In the area selected for the test, 3 replantings had been necessitated by losses from heart rot. Approximately 40 per cent of these also had failed. The causal organism was found to be *Phytophthora parasitica.* The area used was a low one, subject to frequent inundation during heavy rains. It thus presented conditions of maximum severity for a control study. All plants from 4 beds 300 feet long were pulled up. The area was divided as follows: each of the 12 plots in the 2 outer rows was 25 feet long, while the 8 plots of each of the center rows were 36 feet long. There were 2 lots of planting materials: small slips cured 2 weeks, and similar slips cured many months. Some of each were used as nontreated checks, and equal numbers were dipped into freshly prepared Bordeaux 1–0.7–3. They were planted while wet. Thus there were 10 plots of each treatment distributed throughout the experimental area. This entire area was badly flooded by rains twice during the period of observations and more than 80 per cent of the nontreated plants rotted. In spite of these adverse conditions, the plants dipped in Bordeaux were surprisingly vigorous and the degree of control of heart rot was in excess of 70 per cent. Table 8 summarizes the conditions in this experiment after 6 months.

TABLE 8.—*Summary of conditions in control experiment 4, after 6 months*[a]

Treatment	Plants treated	Plants rotted	Control
		Per cent	*Per cent*
A R.C. Nontreated check[b]	531	80.44	
B R.C. Bordeaux dip 1–0.7–3	578	19.97	74.95
C O.C. Nontreated check	588	84.99	
D O.C. Bordeaux dip 1–0.7–3	575	24.78	69.65

[a] Odds indicative of the significance of differences between the treatments are: A–B, >1 to 10,000; A–C, 1 to 2.75; A–D, >1 to 10,000; B–C, >1 to 10,000; B–D, 1 to 9.26; C–D, 1 to 10,000. To be significant odds should exceed 1 to 25.

[b] R.C. indicates recently cured slips; O.C., older cured slips.

The distribution of plots was such that statistical analysis could be obtained by using Student's method for paired observations (10). Results of this analysis (footnote a, table 8) demonstrate that the are no significant differences between slips of recent curing and slips of older curing, but that differences between any set of treated plots and nontreated plots are overwhelmingly significant.

Experiment 5: In a newly planted 200-acre field at Kemoo, Oahu, 10 per cent of the plants were estimated to have been killed by *Phytophthora parasitica.* Local spots of high incidence of disease were evident, where 50 to 75 per cent of plants in acre areas were killed. One such area, 50 beds wide and 300 feet long, was selected for the control test described below. Provision was made here for comparing (A), dipping slips in Bordeaux 1–0.7–3 and planting while wet, with (B), planting nontreated slips and immediately discharging enough of the same Bordeaux into their hearts to fill the plant and wet the soil thoroughly around the butt of each.

Seventy-five plots of 100 feet long and 2 beds (2 rows each) wide were laid out so that each line included a replication of each treatment. The distribution of plots was such that the 25 replications provided the following varieties and numbers of lateral contacts: A with B, 24; A with check 22; B with check 22. Not all plants in these plots were a part of the experiment. Certain of the original healthy plants were left standing. These were identified by painting a leaf of each with white paint. The test plants were used as replants and were placed where dead plants had been removed.

Table 9 shows the number of plants treated, the percentage of plants in

TABLE 9.—*A summary of conditions in control experiment 5, Kemoo, Oahu, after 6 months*

Treatment	Plants treated	Plants rotted	Control
		Per cent	*Per cent*
A Bordeaux 1–0.7–3 dip	3161	13.8[a]	63.4
B Bordeaux liquid in hearts	3053	26.3	30.2
X Nontreated check	3082	37.7	

[a] Odds indicative of the significance of differences between the treatments are: A–B, $>$ 1 to 10,000; X–A, $>$ 1 to 10,000; X–B, 1 to 713.

each treatment that rotted, and the percentage control effected by the 2 types of applications. Added to this table as a footnote are the odds corresponding to the Z values of Student's tables (9). They show that all differences in table 9 are highly significant.

Experiment 6: The experiments already presented indicate that Bordeaux 1–0.7–3 is the most effective of all fungicides tested. A series of field studies was planned, therefore, to give information regarding the best means of applying it to planting materials. The first of these was located in an area where *Phytophthora parasitica* was abundantly distributed. Five means of applying Bordeaux 1–0.7–3 to slips were compared (Table 10).

The experimental area was laid out in 17 lines of increasing length. Each line, regardless of its length, was divided into 7 equal plots, 2 of which were used for checks, and 1 for each type of treatment; hence there were 17 replications of each treatment and 34 of the check. The plots in row 1 were each 13 feet long, in row 2, 15 feet long. They increased from row to row by an addition of 2 feet, so that plots of row 17 were 45 feet long. The distribution of treatments and check was random.

A very spotted development of disease occurred, the percentage of diseased plants in the nontreated checks varying from 3.4 to 78.7. Such uneven distribution of heart rot made small plots and many replications desirable.

Table 10 summarizes the pertinent data of experiment 6. It presents, for each treatment, the numbers of plants treated, the percentage of plants rotted, and the percentage of control effected. Footnote a of table 10 is

TABLE 10.—*Summary of experiment 6 after 6 months*[a]

Treatment	Plants treated	Plants rotted		Control
		Treated plots	Adjacent checks	
		Per cent	*Per cent*	*Per cent*
A. Bordeaux 1–0.7–3 dip in field	967	3.8	32.86	88.44
B. " liquid in hearts after planting	914	17.42	34.56	49.55
C. " butt spray, dry, top spray	932	12.18	31.75	61.64
D. " dip at trimming bed, plant dry	905	7.55	37.9	80.10
E. " dip as D, spray tops after planting	911	4.61	33.26	86.17
X. Nontreated checks	1892			

[a] Odds indicative of the significance of differences between treatments are: A–X, >1 to 10,000; B–X, >1 to 10,000; C–X, >1 to 10,000; D–X, >1 to 10,000; E–X, >1 to 10,000; A–B, >1 to 10,000; A–C, >1 to 10,000; A–D, 1 to 95; A–E, <1 to 2; B–C, 1 to 20; B–D, >1 to 10,000; B–E, 1 to 5,000; C–D, 1 to 600; C–E, 1 to 1,000; D–E, 1 to 155.

a brief summary of statistical analysis of the results prepared by Student's method for paired observations (10).

From table 10 it is apparent that the several methods of applying Bordeaux 1–0.7–3 may be arranged in the following series of decreasing efficiency: (1) treatment A, dipping in the field; (2) treatment E, dipping planting materials at the trimming bed and spraying tops after planting (table 10 shows no significant difference between treatments A and E); (3) treatment D, dipping at trimming bed and planting dry; (4) treatment C, spraying of both butts and tops; (5) treatment B, applying the liquid to the hearts of the planting materials after they have been planted.

Experiments 7 and 8: Another set of experiments to test various means of applying Bordeaux 1–0.7–3 was installed on Oahu as an integral part of field plantings. Two upland sites were selected in areas of excessively high rainfall, where *Phytophthora cinnamomi* caused heart rot in prior plantings. Rainfall in normal years was known to be in excess of 100 inches. During the first 6 months of the tests described below, 63.12 inches of precipitation occurred. During one 24-hour period 15.5 inches of rainfall, and during a second similar period 12 inches fell.

The probability that a single application of Bordeaux would provide enough fungicide to protect susceptible plants for a period of months under such conditions seemed slight. Provision was made, therefore, to determine the number of applications necessary to a high degree of control. Duplicate tests were installed at Opaeula and Waimea, Oahu. Bordeaux 1–0.7–3 was used according to 5 schedules (Tables 11 and 12).

TABLE 11.—*Summary of control in experiment 7*

Treatments	Plants treated	Plants rotted		Control	Odds[a]
		Treated plots	Adjacent checks		
		Per cent	*Per cent*	*Per cent*	
A. No dip; 3 liquid applications	1389	3.05	19.22	84.16	1249
B. Dip; 1 liquid application	1394	3.51	17.75	80.23	525
C. Dip; 2 liquid applications	1413	2.17	22.32	90.28	713
D. Dip butt only	1264	3.21	13.33	75.92	587
E. Dip entire plant only	1301	3.16	19.14	83.49	587
X. Nontreated check	4061				

[a] Odds are the values from Love's modification of Student's table (9) corresponding to the computed Z values.

The liquid applications were made from a portable spray tank, using no nozzle, (1) immediately after planting, (2) after 5 weeks, (3) after 4½ months—4 days after the 15-inch rain.

TABLE 12.—*Summary of control in experiment 8*

Treatments	Plants treated	Plants rotted		Control	Odds[a]
		Treated plots	Adjacent checks		
		Per cent	*Per cent*	*Per cent*	
A. No dip; 3 liquid applications	1453	3.97	27.86	85.76	9,999
B. Dip; 1 liquid application	1345	3.37	32.40	89.60	4,999
C. Dip; 2 liquid applications	1331	2.28	23.08	90.13	> 10,000
D. Dip butt only	1426	5.90	24.72	76.14	3,332
E. Dip entire plant only	1356	2.75	21.07	86.95	> 10,000
X. Untreated check	4197				

[a] Odds are the values from Love's modification of Student's table (9) corresponding to the computed Z values.

In both tests 8 replications of each treatment and 24 of the nontreated check were used. Each plot was 38 feet long and 4 rows (2 beds) wide. Each type of treatment was distributed throughout the experimental area and was in contact with nontreated check plots. Data from the 2 experiments are summarized in tables 11 and 12.

Effect of Fungicides on Root Failure Caused by Heart-rotting Organisms: Phytophthora cinnamomi is an active cause of root failure of pineapple plants as well as a cause of heart rot. Observations were made, therefore, to determine the action of fungicides, used for controlling heart rot, on the development of subsequent root-failure symptoms.

Plots of experiment 1, described above, were used for these observations. Five months after the plants had been treated all were examined and were grouped into 4 classes of varying vigor. The class divisions were arbitrary. Plants of class 4 had been removed from the field prior to the counts. Class 3 included plants that were yellow, stunted, and suffering from root failure. The very strong, prominently vigorous plants were tabulated as class 1, and all remaining plants, which were neither very good nor very poor, were placed in class 2. The figures thus obtained revealed a noticeable effect of the various fungicides upon the growth of the plants. All treated plots, regardless of the fungicides used, with the exception of copper carbonate dust, were better than nontreated plots. The indication is that, contrary to being detrimental to the growth of the host plant, the fungicides were beneficial. These observations are presented as table 13.

After 1 year, observations of the plots were again made. Root failure had caused the collapse of so many plants in all treated and nontreated plots alike that no counts were made. From the standpoint of the produc-

TABLE 13.—*Distribution of plants of various degrees of vigor in treated and check plots of experiment 1. Conditions after 5 months*

Fungicide	Percentage distribution			
	Class 1 Very good	Class 2 Fair	Class 3 Very poor	Class 4 Heart rot
Niagara colloidal sulphur	78.89	18.27	2.54	0.3
Ferrox sulphur	78.63	19.13	1.64	0.6
Fungi Bordeaux	77.11	18.49	4.00	0.4
Lime sulphur	63.12	26.55	8.43	1.9
Bordeaux 1–0.35–13.5 + resin	61.48	28.60	9.92	3.8
Bordeaux 1–0.7–6	61.36	28.41	7.53	2.7
Copper carbonate 1–12	59.80	28.33	10.57	1.3
" " + lime 4–1–48	55.89	33.54	7.37	3.2
Bordeaux 1–0.7 –13.5 + resin	55.89	26.72	11.79	5.6
" " no resin	53.92	30.25	20.93	4.9
" 1–0.35–13.5 " "	53.23	26.69	13.38	6.7
Ialine sulphur 1–3	49.84	20.71	10.85	18.6
Copper carbonate + lime 4–1 (dust)	48.19	42.00	9.81	.....
Bayer Special II–6 dust	41.45	31.37	23.78	3.4
Check (slips)	40.91	21.64	14.45	23.0
" (crowns)	29.00	20.00	3.40	47.6
Copper carbonate dust	0.00	0.00	100.00	0.0

tion of marketable fruits, the entire experimental area was a loss. If such collapse were certain to occur in *Phytophthora cinnamoni* areas the use of any material to protect young plants from heart rot would not be justified. Experienced growers agree that while such losses may be anticipated in years of bad weather, they may be absent in good years. Clearly, the control of root rot caused by *Ph. cinnamomi* is a problem separate from the control of heart rot caused by the same organism. The use of Bordeaux dip in such areas is a matter to be determined from field records over a period of years. At present no such records are available. Plants protected from heart rot in *Ph. parasitica* areas mature fruit and are not subject to root failure of the type found in *Ph. cinnamomi* localities.

DISCUSSION

Plot Distribution and the Analysis of Data

To provide a fair test of the relative effectiveness of several competing fungicides for controlling soil-inhabiting organisms, such as the heart-rot pathogens, the plots of each treatment should be so distributed that the sum of the values of soil infestation for all replications be equal between treatments. Such an arrangement of plots was impossible in the present application owing to the absence of methods for measuring the distribution

of the pathogen. An effort was made, therefore, to so distribute plots that heterogeneity arising from sources other than the treatments themselves could be evaluated.

Experiments representative of several types of plot distribution are discussed above: Random distribution of one-line plots of each treatment and nontreated checks with few replications; random distribution of part-line plots in which the numbers of replications were increased; arrangement of treatments so as to insure many lateral contacts between the treated plots and between the treated and nontreated plots; and a modified Latin Square. Experiments of the first 2 types unfortunately did not provide an adequate number of replications in the one case or a proper distribution of checks in the other to make the results statistically significant. The results of experiments 1 and 2 must, therefore, be regarded as approximate only, but nevertheless indicative of major differences in the effectiveness of the several treatments.

The Latin Square permits a mathematical reduction of results in cases of regular soil heterogeneity (14). Experiment 3 of the present paper was laid out according to the requirements of the Latin Square, provision being made, however, for 2 sets of nontreated checks. Each set of checks was regarded as a separate treatment for the purposes of analysis by Student's method (14); hence a check was provided on the validity of applying the Latin Square to the class of irregular soil heterogeneity present in the experimental area. It does not follow, however, that equally significant results would be obtained from the Latin Square in all heart-rot areas. Provision must always be made for adequate checks of the installation, as a whole.

In an analysis of the Latin Square comparisons are made between groups of plots rather than between single plots, and any set of treatments is just as "close" to any other set as it is to the nontreated check. The opportunity for validly examining apparent differences between sets of treatments is thus enhanced. This method also permits a more adequate evaluation of heterogeneity from all causes beyond the treatments themselves than any other plot distribution known. These features make the Latin Square the desirable arrangement to be used. Where it cannot be used, the plots should be arranged for analysis by the method of paired observations.

Host Factors Influencing Control

In the experiments detailed above, indications of different susceptibilities of various classes of planting materials were evident.

A comparison of the relative susceptibilities of crowns and slips was made in experiment 1. Approximately 800 nontreated crowns were dis-

tributed in 10 equal plots throughout the experimental area. Ten similar areas of nontreated slips were contiguous with the narrow dimension of the plots of crowns. Other plots of slips were adjacent to the long dimension of the areas. In all, 16 plots of slips were located for comparison with the 10 plots of crowns. When changes no longer occurred, 19.39 per cent of the slips and 55.54 per cent of the crowns were diseased. Only 35.5 per cent as much rot developed in the slips as in the crowns. It is clear from these figures, and from other observations not presented, that nontreated crowns should not be used where heart rot occurs.

Preliminary indications from experiment 2 were that nonstripped crowns were less susceptible to heart rot than stripped. Control effected by treated crowns of the former type was 84.4 per cent contrasted with 75.6 per cent for treated crowns of the latter type. While these differences are not statistically significant, they agree with greater differences noticeable in nontreated crowns of the 2 types shown in plantation experiments not reported here. Data regarding the effect of stripping on the susceptibilities of slips or shoots to heart rot are not now available.

Control Effected by Sulphur Preparations:

Experiments 1, 2 and 3, detailed above, incorporated the use of Ferrox sulphur, Niagara colloidal sulphur, Ansul wettable sulphur, and Ialine wettable sulphur. A great spread in the effectiveness of the sulphur fungicides in the different experiments is noticeable. In experiment 1 Ferrox sulphur and Niagara sulphur gave a control of 97.13 per cent and 97.18 per cent, respectively. In all subsequent tests sulphurs exhibited little efficiency as agents in the control of heart rot. Thus in experiment 2 the control was −8.19 per cent for Ferrox sulphur and −11.78 for Niagara sulphur; in experiment 3, 48.53 per cent for Ferrox sulphur and 40.45 for Ansul wettable sulphur.

Experiments 1 and 3 were in areas where *Phytophthora cinnamomi* was the pathogen; experiment 2 where *Ph. parasitica* was the pathogen. No explanation can be made at this time to account for the demonstrated differences in effectiveness of the sulphurs in the above experiments. It is interesting to note that less than two grams of the fungicidal dusts were added to each plant in experiment 1, where control was 97 per cent. At the site of experiment 2 an application of 800 pounds of wettable sulphur per acre to the soil, an equivalent of 36 grams per plant, gave only 30.3 per cent control.

There is no apparent correlation between the quantity of rainfall and control in the several areas. Hydrogen-ion and temperature data are not available for all the locations.

Control Effected by Bordeaux Dip:

Bordeaux dip was the one treatment used in all the experiments reported above. Figure 4 graphically presents for each experiment the contrasts existing between the percentage of plants rotted in plots treated with Bordeaux dip and in contiguous nontreated plots. It shows strikingly the beneficial effect of the fungicide. It also emphasizes the variability in the degree of control experienced in the several experiments.

The areas in which the above tests were installed were selected because they represented extremes in conditions favoring heart rot. The control experienced in them is even less, therefore, than may be anticipated in areas of average losses.

Bordeaux, used as a dip, has been more desirable than any other treatment used to control heart rot in the present studies. It combines the qualities of effective control, ease of application, low cost, and absence of toxicity to the pineapple plant. As seen in table 1, wide fluctuations in the ratio of lime to copper sulphate, and in the concentration of the Bordeaux are possible without ill effects to the plants treated. This feature makes Bordeaux especially desirable for use in the fields, where accurate weighing of the ingredients is not always possible.

Control Effected by Copper Carbonate:

Copper carbonate, although effective in controlling heart rot, causes undesirable burning of the plants treated. In experiment 1, detailed above, many of the plants treated with copper carbonate as a dust or as an aqueous suspension failed to take root or to grow even after one year. Those that did grow were smaller and less vigorous than plants in adjacent plots treated with other preparations. Burn appeared at all points where the plants were injured.

Limited tests have shown that the burn from copper carbonate can be largely eliminated by the addition of hydrated lime in the ratio of 4 parts of carbonate to 1 of lime.

Dusts Compared with Liquid Fungicides:

Under the conditions of tests to date, liquid fungicides seem more desirable than dusts. In windy regions appreciable quantities of the dusts are soon removed. Wet planting material cannot be used safely with dust applications because of the caking of destructive quantities of the materials upon it. Liquid preparations, in contrast, have been very lasting in windy areas and in the regions of high rainfall. No appreciable difference has been noted in burning or adherence of liquid treatments when dry or slightly wet planting materials were used.

Dipping Compared with Other Methods of Applying Liquid Fungicides:

From the experiments detailed above, it is evident that a single application of Bordeaux 1–0.7–3, if applied by completely immersing the planting material in the fungicide, protects susceptible plants better than larger amounts of the same or different fungicides applied in other ways.

In experiment 5, reported above, a comparison was made of applying Bordeaux, as a liquid, into the hearts of newly set plants with dipping planting materials in the same material before planting. Although 3 times as much fungicide was required to treat the plants by filling the hearts with liquid as by dipping before planting, the control experienced by the former treatment was less than 50 per cent of that effected by the latter.

From table 10, which summarizes the pertinent data from experiment 6, it is evident that Bordeaux applied as a dip is more effective than Bordeaux of the same strength applied in other ways. Experience gained during the installation of this experiment and of the 2 Oahu tests, discussed above, recommends dipping of the whole plant, for 2 reasons: A higher degree of control is obtained, and less labor is required than for dipping only the butts of plants. There is no appreciable difference between the incidence of disease in plots of slips planted while the fungicide was wet and in plots planted after the Bordeaux had dried. The indications are that dipping may be done at the trimming bed if treatment there is thorough; but, because of the extra care required to assure success when treatments are applied at any considerable distance from the field, it is recommend that dipping be done as near the place of planting as possible.

In experiments 7 and 8, treatment A provided for 3 liquid applications, spaced over a period of weeks, to the hearts of newly set plants. For treatment A nearly 9 times as much fungicide was required as for treatment E, the standard complete dip, yet the latter treatment was as effective in controlling heart rot as the former. Obviously, dipping is the desirable method to use.

The use of stickers did not appear to alter significantly the percentage control by fungicides with which they were used, but did increase their toxicity to the host plant. Their use is not recommended.

The significance of different methods of applying the fungicide for the control of heart rot in relation to the epidemiology of the disease is considered elsewhere (12).

Control Recommendation:

For the control of Phytophthora heart rot of the pineapple the use of a Bordeaux dip 1–0.7–3 is recommended. Only the most vigorous slips of recent curing should be treated. These should be wholly immersed in the

freshly prepared fungicide, preferably in the field where they are to be planted. They may be planted either before or shortly after the fungicide has dried upon them. If slips are not available, crowns may be used. Well cured, nonstripped crowns have given the best control for this class of planting materials. Their use is not unreservedly recommended, however, because of their greater susceptibility to yellow spot, a virosis, and possibly to other diseases.

Records kept for the experiments described in this paper show that the materials required to treat 3,000 slips cost $2.00 or approximately 0.07 of a cent per slip. Crowns can be treated for less.

The fungicide used in these studies, prepared according to the procedure given below, was selected after preliminary studies only. It is recognized that additional investigation of means of preparing the Bordeaux may increase the degree of control obtained. To date, the best preparation has resulted from the use of crystalline copper sulphate and fresh Waianae hydrated lime. The copper sulphate was dissolved in one-half the quantity of water required for the complete fungicide. Shortly before the Bordeaux was to be used, the hydrated lime was thoroughly mixed with the remaining water in a separate container. While stirring the copper sulphate solution the lime suspension was poured into it. The mixture was thoroughly stirred both before and during treatment of the pineapple planting materials. The above procedure for preparing the fungicide is recommended until better methods have been defined.

Since the incidence of heart rot is dependent upon the environment as well as upon other factors listed in an earlier part of this paper, original plantings should be treated only in areas where repeated losses in successive plantings have been demonstrated. In the absence of definite information covering a number of years, and under ordinary field conditions, only replants should be dipped in Bordeaux.

Heart rot was segregated above into 2 classes, that occurring in very young plants soon after planting, and that occuring in more mature plants. The control measures discussed in this paper are planned primarily as a means of limiting the loss of replants resulting from heart rot of the first and more usual class. Means of controlling losses from the second class of disease remain to be studied.

SUMMARY

Heart rot of the pineapple plant in Hawaii is caused by three species of Phytophthora: *Ph. cinnamomi, Ph. parasitica* and *Ph. palmivora.* Experiments in naturally infested areas involving 2 of these fungi have shown that Bordeaux 1–0.7–3 as a dip is an effective and economical control. This formula was selected from 67 formulae of 22 liquid and 12 dry fungicides

tried. Bordeaux 1–0.7–3, applied by completely immersing planting material in it has given better control than larger quantities of the same or different fungicides applied in other ways. A single application in 8 separate experiments has given the following control under conditions extremely favorable to the development of the disease: Average control 80.29 per cent, range 63.40–90.95 per cent; disease in adjacent nontreated plots, average 44.8 per cent, range 19.14–83.07 per cent: The total concentration of Bordeaux may be doubled, or either constituent varied 50 per cent without injuring plants treated. The cost of materials is approximately $7.00 per acre of 10,000 plants. Specific recommendations for the use of this treatment have been made.

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SNOWMOLD OF TURF GRASSES AS CAUSED BY FUSARIUM NIVALE[1]

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INTRODUCTION

Winterkilling of turf grasses presents an important problem in the maintenance of fine turf, particularly in the northern States. Winterkilling is a general term that has been used in referring to injuries to turf caused by poor drainage, to drying of grass due to cold dry winds, late freezes after the grass has begun to grow in the spring, and to fungous disease. The disease, of parasitic origin, occurs during the winter and early spring and is called snowmold because of its association with snow. The purpose of this study was to determine the cause of this disease and investigate the relation between the host and the parasite. It was also desired to determine the climatic and soil conditions that favored its development, influences of cultural practices, and to work out some means of control.

GEOGRAPHIC DISTRIBUTION AND HOST RANGE

Snowmold is prevalent in Europe, where it occurs on winter grains and lawns in Germany, Denmark, Norway, Sweden, and Russia. The destruction of grain was recorded by Knutberg (7) in 1768. Some of the early studies of the disease and its causal organism were made by Fries (2), Unger (19), Pokorny (13), Fuckel (3), and Sorauer (15, 16, 17).

In North America it is prevalent on golf courses and lawns in the northern tier of States and in Canada. It is most important in Wisconsin, Minnesota, Michigan, and Canada, but also occurs with severity in other States in the same latitude. In the States south of the northern tier it is frequently found in a few places but causes less damage. It has been reported as far south as Virginia.

DESCRIPTION OF THE DISEASE

Most of the damage to turf in this country occurs on putting greens of golf courses, but it also occurs on fairways and lawns. When it occurs on

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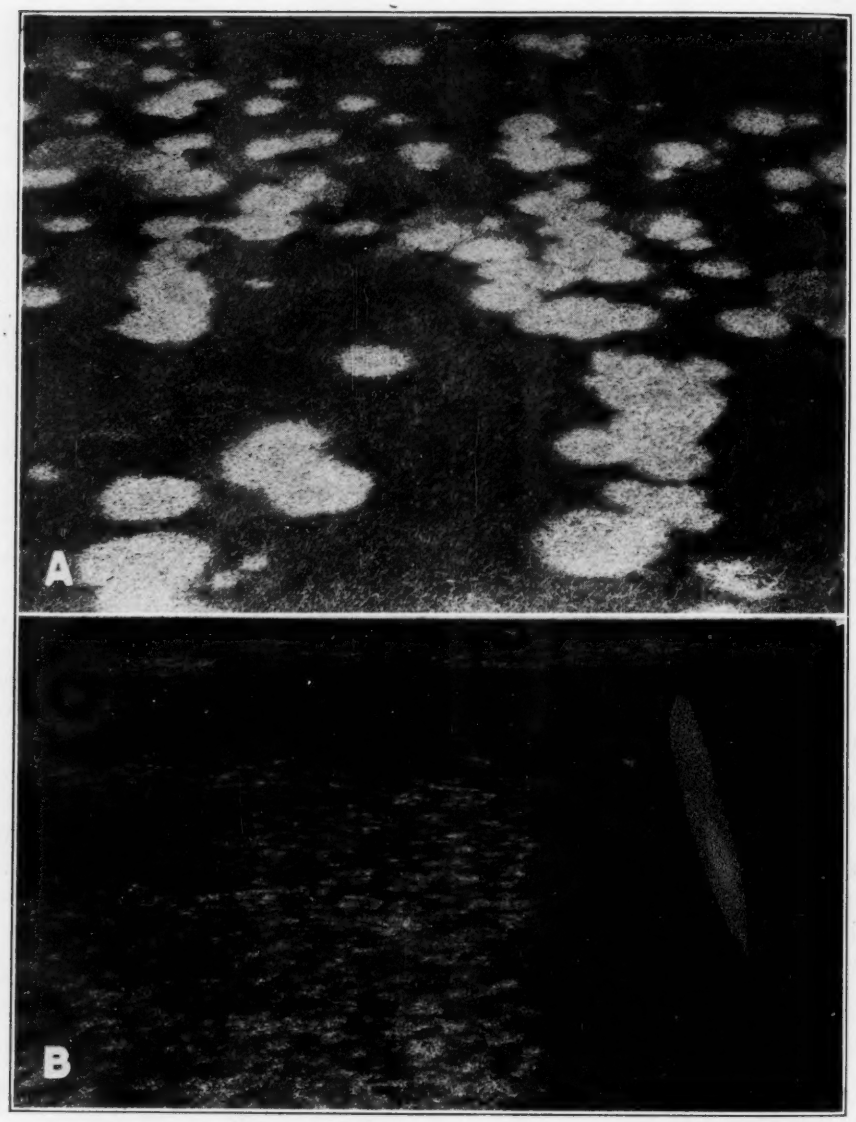

FIG. 1. A. Damage to green at Grand Rapids, Mich., caused by *Fusarium nivale*. The lighter areas are snowmold patches. Photographed on April 3, 1928. The grass on this green did not recover fully until midsummer. B. Plots of putting-green turf showing control of the disease with corrosive sublimate. The treated plot was laid out in the shape of an L and the diseased area on the left and in the background was nontreated. The fungicide was applied in the fall and the photograph was taken in April of the following year.

putting greens it may kill the turf outright; but, even when the disease is not serious enough to kill the grass, it often takes a long time for the grass to recover. Weeds are apt to develop in the weakened patches because of the absence of competition from the grass.

The disease is noticeable as soon as the snow melts, when dead areas are found in the turf. These areas usually are irregularly circular, from a few inches to a foot or more in diameter and, in severe cases, may run together and cover large areas (Fig. 1, A). The patches usually are whitish gray, the leaves have a bleached appearance and, when wet, feel slimy. These patches develop under the snow while it is melting and at that stage they are often covered with an aerial mycelium (Fig. 2). When the conditions remain long favorable for its development, the aerial mycelium may be so abundant that the leaves are matted together, forming a thick layer

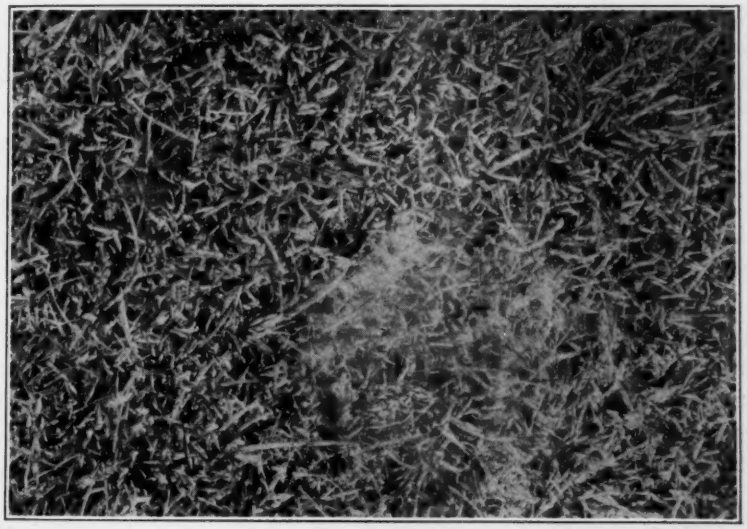

FIG. 2. Fluffy mycelium of the fungus growing over the leaves in the first stage of a snowmold patch.

over the patch. Under this layer the fungus may continue to develop after the grass has dried and the turf may be killed. When the patches are exposed to the sunlight a pinkish cast often becomes apparent on the leaves, due to a change in color of the mycelium. Light brown areas of turf killed by other agents are readily distinguished from snowmold injury. Patches not having an abundant growth of mycelium are frequently not killed but will recover slowly. The organism usually attacks only the leaves, but when conditions are favorable for a long period it spreads into the stems and crowns.

The disease occurs on Kentucky bluegrass, *Poa pratensis* L., annual bluegrass, *P. annua* L., redtop, *Agrostis alba* L., creeping bent, *A. palustris* L., velvet bent, *A. canina* L., Colonial bent, *A. tenuis* Sibth., red fescue, *Festuca rubra* L., rye, *Secale* spp., wheat, *Triticum* spp., and barley, *Hordeum* spp.

CAUSAL ORGANISM

Snowmold occurring on winter grains in Europe has been shown to be due mostly to *Fusarium nivale* (Fr.) Ces. (*Calonectria graminicola* (Berk. and Brm.) Wr.) and in part to *F. culmorum* (W. G. Sm.) Sacc. Schaffnit (14) in examining material from the field found *F. nivale* in 28 out of 29 samples and *F. culmorum* in the one exception. Lindfors (8) examined at least 200 samples and found *F. minimum* (*nivale*) present in all of them. Schaffnit (14) was able to produce snowmold-like symptoms by inoculating with *F. culmorum, F. herbarum* (Corda) Fries, *F. didymum* Harting, *F. avenaceum* (Fr.) Sacc. and *F. lolii* Smith as well as *F. nivale* on etiolated or weakened plants. Lindfors (8) believed that *F. minimum* (*nivale*) was the most important and that the others may play a minor part in the occurrence of the disease. Atanasoff (1) inoculated pots with *Gibberella saubinetii* (Mont.) Sacc., *F. culmorum, F. culmorum* (W. G. Sm.) Sacc. var. *leteius* Sherb., *F. avenaceum* (Fr.) Sacc., *Helminthosporium sorokinianum* Sacc. and two other undetermined *Helminthosporium* spp. and produced conditions similar to those occurring in the field but apparently he did not make his inoculations when the temperature was near the freezing-point, the optimum for true snowmold.

Numerous inoculations have been made in moist chambers maintained at temperatures between 0° and 5° C. with *Fusarium nivale, F. culmorum, F. avenaceum, F. solani* Ren. and Bert., *Gibberella saubinetii,* and numerous other undetermined fungi isolated from diseased grass leaves. Of all these species only *F. nivale* proved pathogenic. Other soil fungi are often found associated with *F. nivale* in diseased patches of turf and are, at times, more conspicuous than the primary organism. In pure-culture inoculation, however, these do not produce the disease.

Cultures of *Fusarium nivale* that were used in inoculation work were isolated from diseased leaves of grass taken from patches showing symptoms of snowmold. The diseased leaves were placed in Petri dishes on water agar and transfers were made from the colonies that developed. The cultures were then grown on potato-dextrose agar. Isolations were made with material from Madison, Wisconsin, Grand Rapids, Michigan, Chicago, Illinois, and from Minneapolis and Duluth, Minnesota. Single-spore cultures were made by the dilution method after the cultures began to sporulate.

Cultural Studies: The cultures were grown on corn-meal agar, oat-meal agar, potato-dextrose agar, and grass clippings. On corn-meal agar there was a scant aerial, colorless, and sterile mycelial growth. A very abundant mycelial growth developed on oat-meal agar and spores were produced in slimy salmon-color masses, often covering a large part of the surface of the agar. On potato-dextrose agar there was an abundant, white, fluffy, spore-bearing mycelium. On sterile grass clippings, the fungus covered each leaf with a fluffy white mycelium that in a few cases produced spores.

When the cultures were kept in darkness from the time they were isolated, there was no spore production, but when transferred to diffuse light, especially in a moist chamber, sporodochia were formed and great masses of spores developed. After the spores were once produced in light, the cultures continued to sporulate on subsequent cultures, when transfers of spores were made. Light also changes the color of the mycelium from white to a pale pink, which was demonstrated in Petri-dish and test-tube cultures and on mycelium on potted grass plants.

Schaffnit (14) found that *Fusarium nivale* grew between temperatures from 0° to 30° C., with an optimum of 22° C. Lindfors (9) and Tu (18) also found that the optimum temperature was 22° C. In the studies herein reported 3 cultures of *F. nivale,* isolated from diseased leaves from snow-mold patches, were grown in constant-temperature chambers from 2 to 36° C. The cultures were grown in Petri dishes containing 10 cc. of potato-dextrose agar. Transfers of mycelium were made from young Petri-dish cultures, and the hourly rate of growth was calculated after 72 hours of growth.

Figure 3 shows the hourly growth rates at the various temperatures. The 3 cultures agree generally in their growth curve. The amount of growth at 2° C. indicates that the fungus is adapted to low temperatures. In general, the optimum appears to be about 20° C. and all of them grow as well or better at 16° as at 24°. There is a sharp drop in the curve above 24° and, although there was some growth at 32°, there was none at 36°.

Infection Experiments: The pathogenicity of the organism was proved by inoculation of potted grass plants. All of the grasses were grown in autoclaved pots and soil. The seed was treated with 1–320 Formalin for 15 minutes. The grasses were grown in the greenhouse and clipped from time to time, so that the plants were usually about 2 inches high.

The strains of the fungus were grown on sterilized grass clippings in culture tubes until the mycelium had grown throughout the clippings in the tube. A portion of the clippings was then transferred to the center of a pot of grass and held about 1½ inches above the surface of the soil by the leaves. The pots were then placed in moist chambers. Most of the inoculations were placed in a chamber kept from 0° to 5° C., since best results

were obtained at that temperature. Successful inoculations were made on creeping bent, redtop, Kentucky bluegrass, fescue, barley, wheat, rye, and oats. All of the inoculations were made on seedlings, except creeping bent, where old sod was brought into the greenhouse and potted.

In most cases infection of the leaves had taken place within 2 days after inoculation. When the amount of moisture in the chamber was decreased the infection was slight and the spread of the organism from leaf to leaf was slow. The mycelium of the organism spread quickly, when the atmosphere of the chamber was foggy, until all the leaves of the grass were covered with mycelium, as in figure 4.

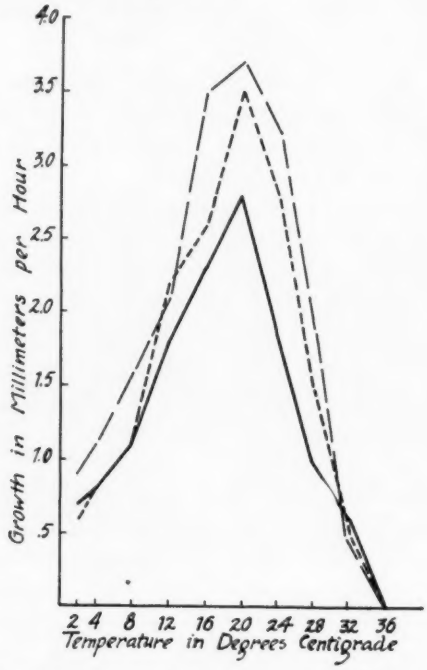

FIG. 3. Effect of temperature on the rate of growth of 3 strains of *Fusarium nivale* on potato-dextrose agar. Note their essential likeness with a rather slow rise up to 10°, then more rapid up to the optimum of about 20°, with a rapid fall above 24°. This is in contrast with the most culturable parasitic fungi, which show an optimum at about 24–30° C.

Inoculations were placed in chambers kept at temperatures from 0–5°, 5–10°, 10–15° and 15–20° C. The best results were obtained in the 0–5° chamber. The mycelium spread quickly and was abundant and fluffy. In the 5–10° chamber there was also an abundant growth of mycelium and a rapid spread of the fungus over the leaves. At 10–15° the development was much slower and the mycelium was less abundant. At 15 to 20° there

was a very small amount of infection near the inoculum; there was no aerial mycelium and, after 3 weeks, very slight spread of the organism.

Histological Studies: Material obtained from plants of Kentucky bluegrass, redtop, and creeping bent, inoculated with pure cultures in the greenhouse, was used to determine the method of penetration of the organism and the relation of the fungus and host tissue. Upon examination of prepared slides, it was found that the mycelium developed along the outside of the leaf and, when a hypha reached a stoma, it enlarged at the terminal end and one or more infecting hyphae developed down through the stomatal opening into the intercellular spaces. (Fig. 5, A). No direct penetration through the epidermal cells was observed until after the cells within the leaf had collapsed and there was an abundant development of mycelium. The

FIG. 4. Inoculation of pot culture of Kentucky bluegrass with *Fusarium nivale*. A. Control. B. Diseased plants with an abundance of aerial mycelium.

mycelium developed faster along the surface of the leaf than it did within the leaf tissue, so that new infections through stomata took place along the entire length of the leaf until it became wholly infected. When temperature and moisture conditions were not favorable for abundant aerial mycelium, the infection of the leaf was slower because less mycelium grew along the surface.

The growth of the organism in the tissue is intercellular with much branching until numerous hyphae extend in all directions. Later the cells collapse and the fungus almost fills the space occupied by the collapsed cells. The fungus then penetrates into the vessels.

It was observed that when a leaf is badly diseased, sporodochia develop through the stomata in rows. An abundant growth of mycelium at first

occurs through the stomata and later develops into sporodochia that bear great masses of spores on club-shape sporophores (Fig. 5, B). Most of the spores become detached during the preparation of the slides. In some

FIG. 5. A. Infection of a leaf through a stoma. Note the enlargement of the end of the infecting hypha and the intercellular course of the invading hyphae. B. Sporodochium as it occurs on a stoma. The guard cell of the stoma is located just below the sporodochium.

cases the sporodochia become so large that they cover a considerable surface of the leaf, so that 2 or 3 stomata are involved. After the leaf is badly

diseased, penetration through the epidermis takes place in great abundance, nearly all of the parenchyma cells collapse and only the vessels are left intact.

INFLUENCE OF WEATHER ON OCCURRENCE OF DISEASE

Weather conditions play an important part in the development of the disease. Unger (19) reported that snowmold was serious after a wet fall, when snow fell on unfrozen ground. Pokorny (13) also found snowmold very severe on lawns during the spring of 1865, after a winter of continuous snow cover and a slow thaw in the spring.

Sorauer (15, 16) stated that the organism grew continually during mild winters and stopped only when it freezes. He explained that cavities were formed between the snow cover and the ground and in these cavities there was a saturated atmosphere and low temperature, which was suitable for the development of the fungus.

Hiltner and Ihssen (6) found snowmold worse on northerly slopes where the snow melted slowly in the spring. Mortenson (12) observed that the disease was worse after wet summers, when the moisture in the ground was abundant as the snow fell.

In Minneapolis, Minn., in the fall of 1927, when snow fell before the ground was frozen, severe attacks of snowmold were found on some of the putting greens the next spring. The same was true that year in Grand Rapids, Mich., where the snow melted before the end of the year and the disease was apparent in the latter part of December.

Deep snow, however, is not always necessary for the development of the disease. During the winter of 1927–28 at Madison, Wis., many greens on some of the courses were bare much of the time and snowmold was present on these greens. There was much cold and cloudy weather, and apparently there was sufficient soil moisture and atmospheric humidity to promote growth of the organism.

Sunlight and dry winds soon stop the development of the organism after the snow has disappeared. Where there has been an abundant growth of mycelium and the leaves are matted together, air and sunlight are kept out, moisture is held in the turf, and the organism continues to grow and to injure the turf severely. Hiltner and Ihssen (6), Atanasoff (1), and Schaffnit (14) record that the mycelium disappears as soon as the sunlight or air currents come in contact with it and the plants then begin to recover. In turf, where the plants are crowded, sunlight does not penetrate and the fungus may continue to live. Whenever a cloudy day occurs, it may again become active and spread. Greenkeepers have made a practice of brushing these patches to break the mat of leaves and mycelium. This dries the patches and lets the sunlight in, so that the development of the organism is stopped.

During the winter of 1928–29 temperatures were recorded under the snow at Wayzata, Minn. The thermograph bulb was placed under a covering of straw and during the winter was buried under a thick layer of snow. The temperature remained almost constant at 32° F., although the air temperature was as low as – 20° F. In the winter of 1930–31 soil temperatures were recorded at Madison, Wis., where the thermograph bulb was placed just beneath the surface of the turf. When snow was present, the temperature remained nearly constant at 0° C. no matter what the air temperature was. When the snow melted, the soil temperature began to vary with the air temperature. The temperature under a covering of straw was the same as in the uncovered plots when snow was present, so that the covering of straw evidently did not raise the temperature. These temperature records indicate that the development of the organism takes place at about 0° C. This has been borne out by inoculation in the greenhouse where best results were obtained from 0° to 5° C.

INFLUENCE OF SOIL AND CONDITION OF GRASS ON OCCURRENCE OF DISEASE

Condition of the soil may affect the occurrence of the disease in two ways, first, by its influence on the growth of the plants and second, by its influence on the development of the organism. Hiltner (4) found the disease worse on heavy, poorly worked, poorly drained, clay soil, and on soil rich in humus. Hiltner (5) also found that manuring and covering with straw encouraged the development of the organism.

In rich soils there is a very luxuriant growth of the plants, late in the fall, just before the snow falls; and when the grass is fertilized late there also is a marked growth of grass. Plots of putting-green grass fertilized with ammonium sulphate on November 6, 1930, in Chicago, Ill., when observed on December 6, had a very dark green appearance in contrast to the checks, which had not been fertilized and were decidedly brown. Even though the temperature be very low, grass will grow when stimulated by fertilizer and the development of snowmold may be encouraged.

Sorauer (16–17) states that the fungus attacks the leaves mainly because of its thinner epidermis and its rich plasma. Hiltner and Ihssen (6) found that snowmold was worse on fields where the plants were larger and held the snow up from the ground, so that cavities were formed where the fungus could develop. They also found that grazing in the fall reduced the amount of snowmold because of the smaller amount of leaf surface present when the snow fell. Lindfors (8) recommended late seeding, low rates of seeding, and cutting or grazing in the fall, so that the leaf area would not be too great. He also states that when a mat of leaves and mycelium are formed, under which the fungus may continue its development, the plants are severely injured.

It has been the practice of some greenkeepers, especially in the vicinity of Minneapolis, to cover their greens with straw during the winter. In many cases this practice has led to very serious damage from snowmold. Other greenkeepers who fertilize in the fall, or have a great deal of organic matter in their top dressing, have trouble with serious attacks of the disease.

In order to determine what effect fall fertilization or straw covering would have on disease incidence, experiments were made on turf of putting greens during the winters of 1928–29 and 1929–30.

In the fall of 1928 plots of turf were laid out on a green of a golf course near Minneapolis. All combinations of corrosive sublimate, fertilizer, and straw were applied so that the experiment included plots treated with corrosive sublimate; corrosive sublimate and straw; corrosive sublimate and fertilizer; corrosive sublimate, fertilizer, and straw; fertilizer with and without straw; and straw and check plots. The use of corrosive sublimate and calomel as fungicides will be considered farther on.

The corrosive sublimate, at the rate of 3 oz. to 1000 sq. ft., was diluted with a quart of sand and broadcast over the plots. The fertilizers used were activated sludge, manufactured by the Milwaukee Sewage Disposal Commission, and ammonium sulphate. These were applied at the rate of 28 lbs. of sludge and 6½ lbs. of ammonium sulphate to 1000 sq. ft. and were also broadcast on the plots. The straw was applied Dec. 1 in a layer about 2 in. thick.

Table 1 gives the results of this experiment, the figures representing the percentage of the area killed by the disease. These percentages were determined in the spring after the straw had been removed on April 3. From

TABLE 1.—*Results of field experiments on plots of putting-green turf. The figures represent the percentage of turf affected by snowmold*

Materials applied	Minneapolis		Grand Rapids	
	Covered with straw	Not covered	Covered with straw	Not covered
	Per cent	*Per cent*	*Per cent*	*Per cent*
Checks	100	0	50	20
Corrosive sublimate	5	0	20	2
Corrosive sublimate and fertilizer	50	0	30	10
Fertilizer	100	0	90	40
Calomel and fertilizer			70	20
Calomel			30	5

the table it will be noted that when no straw had been applied there was no disease and where straw was applied alone, the grass was completely killed by snowmold (Fig. 6, B). The grass was killed also where the straw and the fertilizer had both been applied. Where corrosive sublimate had been applied only 5 per cent of the area of the turf was injured by disease. In

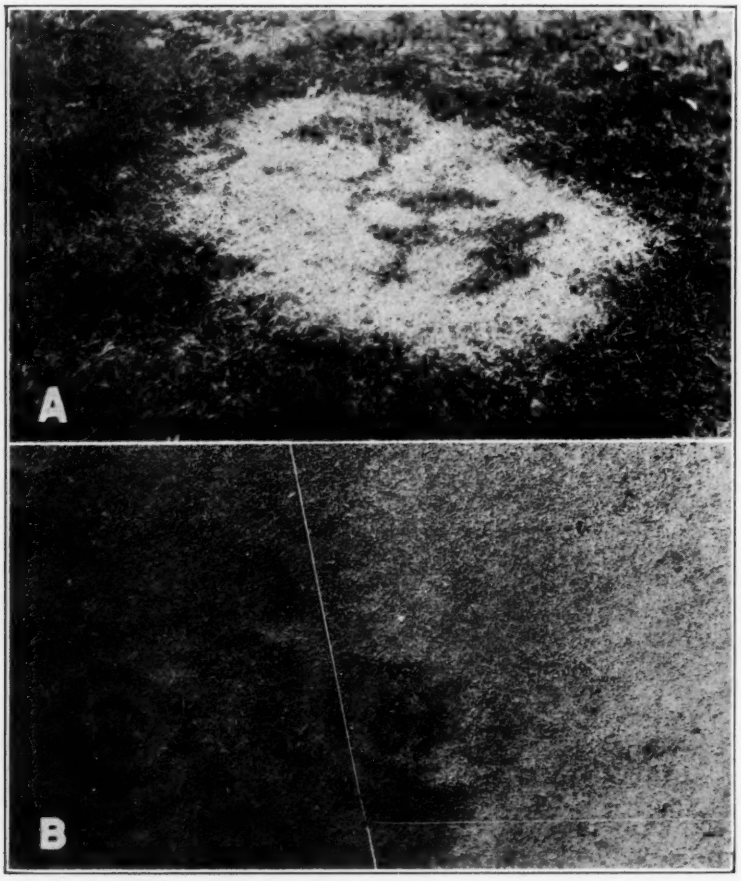

FIG. 6. A. Patch of wild creeping bent with two strains mixed together, one susceptible and the other resistant. The lighter area in the patch is the susceptible strain diseased with snowmold and the darker area is the resistant strain with no disease. B. The turf on the right of line was covered with straw during winter. The lighter area is the dead grass killed by snowmold. The darker area on the left is healthy green grass.

the plots where corrosive sublimate and fertilizer had been applied and covered with straw, 50 per cent of the turf was killed.

This experiment was repeated in the winter of 1929–30 on a golf course near Grand Rapids, Mich. All combinations of corrosive sublimate, fer-

tilizer, and straw also were applied to these plots, and one series was made where calomel was substituted for corrosive sublimate. The corrosive sublimate and calomel were applied at the same rate as in the previous experiment. The fertilizers, activated sludge, and ammonium sulphate, were applied at the rates of 25 lbs. of sludge and 5 lbs. ammonium sulphate to 1000 sq. ft.

The results of this experiment, as given in table 1, show that the efficiency of control by chemicals is greatly reduced by fertilizers and straw covering. There was a more general distribution of disease over all of these plots but none of them suffered so severely as some of the areas of the previous year's experiment. The most severely injured plot was that fertilized and covered with straw, 90 per cent of which was covered with disease. In every case the covering of straw more than doubled the amount of disease and in some cases the disease was increased many times. Fertilizing also more than doubled the amount of disease. Not only do straw covering and applications of fertilizer in the fall increase the amount of disease, but it is much more severe; more of the stolons are infested and the turf recovers more slowly than when the disease occurs where the turf was not fertilized or covered with straw. The results of these experiments show the efficiency of mercury fungicides when applied alone and that this effectiveness may be much reduced by cultural practices.

FIELD EXPERIMENTS ON CONTROL MEASURES

Diseases of grasses in turf have been controlled for the last few years by broadcasting or spraying fungicides on the turf. Monteith (11) was able to control brownpatch, caused by *Rhizoctonia solani* Kühn, and dollarspot caused by *R.* sp., by treating the turf with mercury compounds. The most practical of these have been corrosive sublimate and calomel. For this reason, when a control for snowmold was sought, attention was turned to these chemicals.

Monteith (10) made the first preliminary tests during the winter of 1926–27, when he treated plots of turf on greens of a golf course at Madison, Wis., with corrosive sublimate at rates of 1 and 2 oz. to 1000 sq. ft. These treatments were effective in preventing snowmold in the spring of 1927.

Since then, experiments have been conducted to determine the amount of fungicide necessary to the effective control of the disease. In these experiments, plots of turf were laid out on greens of golf courses at Grand Rapids and Minneapolis. These plots were treated at the rates of 1, 2 and 3 oz. to 1000 sq. ft. The plots were separated by 2-ft. strips of nontreated turf to serve as checks. The chemicals were applied by carefully mixing each with one-half bucket of sand or fine compost and this mixture was broadcast over the plot. The checks were treated with the same amount of sand or compost without the fungicide.

Table 2 shows the results obtained on 4 of the experimental plots. The figures represent percentages of area of the turf affected by disease. The largest rate of application was not entirely effective in most cases. However, where the disease did occur on these plots and where it occurred on greens treated with a 3 oz. to 1000 sq. ft. rate, it was rarely severe and the turf soon recovered. The 2-oz. rate decreased the amount of the disease considerably, and here, too, the severity of the injury was lessened. The 1-oz. rate was not very effective, although it did lessen the severity of the disease to some extent. In the checks the amount of disease was not only greater but the injury to the grass was severe and the turf took longer to recover. Figure 1, B illustrates the effectiveness of control of snowmold with corrosive sublimate.

Golf courses that had been troubled with snowmold for several years have for the past few years been making a practice of treating all of their greens with either calomel or corrosive sublimate and have been successful in preventing serious attacks. Calomel and corrosive sublimate are almost equally as effective and both are used widely by greenkeepers. Some greenkeepers treat only those greens which are likely to be attacked and thus are saved the expense of treating all of their greens.

TABLE 2.—*Results of field experiments on control of snowmold on putting greens on golf courses. The figures represent the percentage of diseased turf. Each series of plots was laid out on different greens*

Series number	Grand Rapids		Minneapolis	
	1	2	3	4
Treatments	*Per cent*	*Per cent*	*Per cent*	*Per cent*
3 oz. Corrosive sublimate	5	5	0	5
3 oz. Calomel	5	20	0	5
2 oz. Corrosive sublimate	40	40	0	20
2 oz. Calomel	20	40	0	20
1 oz. Corrosive sublimate	80	60	20	40
1 oz. Calomel	40	20	40	20
Checks	90	90	80	60

VARIATION OF RESISTANCE TO DISEASE IN STRAINS OF GRASS

It has been observed that there is a difference in the susceptibility of varieties of winter grains, to attacks of Fusarium blight. Hiltner and Ihssen (6), Lindfors (8), and Atanasoff (1) mention this difference in resistance. The same has been observed to be true with different grasses used on golf courses.

The amount of snowmold that may occur on a golf course may be governed by several factors. It may be due to the location of the greens, that

is whether or not they are sheltered from sun and wind and are covered with deep drifts for long periods in the winter. On such greens the snow melts slowly in the spring and poor drainage may make attacks of the fungus more severe. The methods of culture used by the greenkeeper is another factor. If he fertilizes heavily late in the fall or supplies an abundance of organic matter, it is probable that he will be troubled with attacks of snowmold.

The resistance of the grass to snowmold also is a factor in the occurrence of disease on any particular golf course. It has been observed that some strains of creeping bent are very susceptible, while others are noticeably resistant. The Columbia type strains, which make a thin turf, are often severely damaged by numerous patches of snowmold. The turf does not recover in these patches, but must fill in from the edges of the patches when growing weather comes. This type of grass is used on several golf courses in Grand Rapids and Minneapolis, and is one of the reasons why those particular courses are troubled with snowmold. The Washington strain of creeping bent is often attacked by snowmold, but the disease is rarely serious enough to warrant resodding and usually the injury disappears early in the spring. Seaside creeping bent, which is propagated by seed, while the others are propagated by stolons, also is a susceptible strain, and when observed on golf courses it usually is severely attacked by disease. On demonstration plots in Detroit and Grand Rapids, where several grasses are grown in turf in 10′ by 10′ plots side-by-side, the seaside bent has been observed to be severely attacked, while Colonial bent beside it did not have a spot of disease.

Metropolitan bent is resistant to snowmold. On the demonstration plots, where snowmold has appeared on other grasses, little has developed on this strain. At Wayzata, Minn., Metropolitan and an unnamed strain, found on the golf course in 1929, were growing side-by-side. The wild strain had 4 or 5 times as much disease as the Metropolitan strain.

Fescue is susceptible and on some golf courses where fescue greens were planted the grass has been eliminated by snowmold. This is one of the limiting factors in the growing of fescue greens in this country.

On a fairway near Grand Rapids several strains of creeping bent were growing together. Some of these were in large patches and several had been attacked by snowmold. One patch was observed that had a mixture of 2 strains of bent. One of the strains was severely diseased and the other was green. Figure 6, A shows this patch of grass.

DISCUSSION

Hiltner and Ihssen (6) found snowmold worse on strong vigorous plants and on manured soils or where straw had been applied. They recom-

mended grazing the fields in the fall because it reduced the amount of leaf area. Lindfors (8) also recommended reducing the amount of leaf area by later seeding, fall grazing, or cutting. Both investigators explain the increased severity of snowmold on the assumption that larger plants give off more heat because of the greater amount of respiration. However, the temperature under the snow does not vary from the freezing-point and is not higher where there is greater leaf area or where straw has been applied. The greater amount of snowmold under those conditions may be, in part, due to the ease with which the organism can spread from leaf to leaf. When the leaves are abundant and large, the mycelium overgrows the leaves and they become matted together to form a layer over the plants. Under this layer the organism can develop as long as temperature remains low, even after the snow has disappeared.

The large amount of organic matter in putting green turf enhances the disease, and where straw is applied and kept wet by the snow cover, there is an abundance of material on which the fungus can develop saprophytically. From this substratum the mycelium of the organism can spread to the leaves of the grass plants.

The absence of serious attacks of snowmold on winter grains in this country probably is due to the winter freezes that occur before snow falls. In Europe snow falls before many frosts have occurred and before the growth of the plants has stopped. In this country these freezes stop the growth of the plants and the tissue of the leaves becomes mature. When the plants are growing actively and the vegetation is soft and succulent, there is rapid assimilation and respiration. These processes are balanced and as the tissues mature they are slowed and there is a storage of carbohydrates and a loss of water from the cells. Such mature tissue is apparently not susceptible to attacks of the disease. But when the plants are still in an active vegetative state and snow occurs, the elimination of light stops assimilation; respiration continues, so that the carbohydrate reserves are reduced and the tissues may be a better medium for the growth of the fungus. Under these conditions the plants are more susceptible to attacks of the disease. On putting greens it has been apparent that strong growth of grass in the fall encourages attacks of snowmold. This is not due to increased temperature and cannot be explained in all cases by increase in leaf tissue because grass is clipped very short on greens in the fall. Plots treated with fertilizer were cut the same height as the nontreated plots, so that the amount of leaf area was almost the same. It is probable that the greater amount of disease is due to the immaturity of the tissue of the leaves, which makes it more favorable for the development of the fungus than the more mature tissue of plants that are not growing. On putting greens, grass stimulated by fertilizers late in the fall, is not allowed to mature before snowfall, thus making it susceptible to attacks of snowmold.

SUMMARY

Snowmold occurs on winter cereals in northern Europe and on turf grasses in the United States and Canada. It is particularly important on golf courses in the United States and Canada. The disease occurs on turf in the form of irregularly circular patches of dead grass. These appear as the snow is melting and usually are from a few inches to a foot or more in diameter and may run together to cover large areas. The patches are whitish gray or may have a pinkish tinge. Individual plants have a bleached appearance and feel slimy when wet. On golf courses the disease occurs on Kentucky bluegrass, annual bluegrass, redtop, red fescue, creeping bent, velvet bent, and Colonial bent.

Snowmold on golf courses in this country was found to be caused by *Fusarium nivale* (Fr.) Ces. (*Calonectria graminicola* (Berk. and Brm.) Wr.). The organism grows well on oat-meal and potato agar on sterile grass clippings. Spores are formed on sporodochia when the culture is exposed to diffused light. Light changes the color of the mycelium from white to pink. Temperature studies showed that the organism grew at temperatures from 2° to 32° C. with an optimum of about 20° C.

Creeping bent, Kentucky bluegrass, redtop, red fescue, barley, rye, wheat, and oats were artificially inoculated in low-temperature moist chambers. The most rapid and injurious infections took place in chambers kept at 0 to 5° C. At 15 to 20° C. infections were slight and very slow.

The organism was found to enter the leaves through the stomata. Its progress through the tissue was intercellular until the cells began to collapse and then it became intracellular. After the mycelium became abundant in the tissue, sporodochia were formed on the leaf over the stomatal openings so that they occurred in rows on the leaf.

Records of temperatures under the snow showed that the organism grew at about the freezing-point. Observations indicated that climatic conditions greatly influenced the amount of snowmold. The conditions that favored attacks of the disease were: abundant moisture in the fall; snow falling on unfrozen ground; deep snow; and a prolonged cold, wet, spring.

Applying fertilizer late in the fall was found to make attacks of snowmold more serious. Where the soil had abundant organic matter or where the greens were covered with straw there was an increase of the disease.

Field experiments showed that the disease could be controlled by treating the turf in the autumn with corrosive sublimate or calomel at the rate of 3 oz. to 1,000 sq. ft. Applications of mercury fungicide at rates of 1 and 2 oz. to 1,000 sq. ft. were only partially effective.

It was found that there was a difference in the susceptibility to the dis-

ease in the grasses used on golf courses. Strains of grass growing side by side often have very different amounts of disease.

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THE LONGEVITY OF THE LATENT AND VEINBANDING VIRUSES OF POTATO IN DRIED PLANT TISSUE[1]

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INTRODUCTION

A review of the literature reveals only a few references that give definite statements regarding the longevity of the latent and veinbanding viruses of potato when dried in leaf or other plant tissues. It was shown by Johnson (5)[3] that the latent virus of potato was short-lived when separated from the living host. He says, "In most cases the survival is less than 20 days and frequently as low as 10 days. In general the virus survives longer in the drying leaf than in extracted plant juice."

As reported by Vanterpool (14), in his work with streak of tomato, "Portions of diseased plants were air-dried at room temperature until thoroughly dry. . . . In no single case did streak develop, but definite mosaic, usually of a very severe nature, invariably was produced when dried diseased material which had been stored for two, three, and nine months was used."

It was observed by Stover (12) that, "The potato element became noninfective in a short time after the death of the affected tissue, while the tomato element may remain infective for some time."

It was shown by Smith (11) that, "in filtered juice (tomato) Majestic mosaic remains infective for five and a half months, the longest period yet tested. Up-to-Date mosaic, however, was found to be inactive after 12 weeks, the filtered juice having been kept in dull light in paraffin-stoppered tubes."

In 1929, Johnson (6) again points out that the potato viruses are short-lived when placed *in vitro*. In working with several viroses of potato he concludes that ". . . The data indicate, however, that in the case of the crinkle mosaic virus a large part of the virus is inactivated at the end of six hours, and that it is all inactivated between 24 and 48 hours. In the case of rugose mosaic (extracted from potato foliage) and the leaf-rolling mosaic virus, inactivation is apparently not as rapid, but again little or no infection may be expected from the virus after aging from 24–48 hours. The mild mosaic virus appears to be the most sensitive. Aging for even

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[3] Reference made by number to "Literature Cited."

two hours *in vitro* appears to cause inactivation, although in one case a low percentage of infection was secured after aging for four hours. The mottle form of the virus from apparently healthy potatoes (transferred to tobacco) will resist inactivation for ten to twelve days. The rugose mosaic (spot-necrosis) virus from tobacco (transferred to tobacco) will also resist aging several days, consequently differing from its behavior when taken from potato."

In 1930 Samson (10) differentiated 2 viroses of Jimson weed. The milder type agrees with the latent ("mottle" of Johnson) from apparently healthy potatoes. "The more severe type . . . resembles the virus of Johnson's spot necrosis or rugose mosaic. Both of these Jimson weed viruses have survived 80 days aging *in vitro* in tomato juice at 10° C."

It was shown by Doolittle and Blood (2) that 3 distinct forms of tomato streak exist in Wisconsin. The form produced by the combination of tomato mosaic and the juice of the potato has a longevity *in vitro* of about 14 days. One of the other forms lives for at least 180 days.

As reported by Johnson (4) the "'Healthy potato' virus does not retain its infectivity in dried tissue (Table 2)." His experiments showed that dried virus-diseased tobacco, which had been aged for 9 months, 27 days, and 16 months, 7 days, respectively, gave negative results. He also points out that the mixed virosis of the latent ("Healthy potato") virus plus veinbanding was noninfective when dried for a similar period of time. He further showed that with the veinbanding virus "The infectivity is destroyed by drying at room temperature (Table 2)." The veinbanding virus was not recovered from extract of dried virus-diseased tobacco that had been aged for 9 months, 27 days and 17 months, 8 days, respectively.

Miss Jarrett (3) concludes that "glasshouse" streak differs from experimental streak (tobacco mosaic plus the latent virus) in that it responds to aging, *in vitro,* closely to Johnson's tobacco virus 1 and differs from potato mosaic in that the latter loses its power of infection after 6 months' storage *in vitro* in tomato or tobacco extract. She further states, "Extracts from plants which have been inoculated with two viruses, viz., experimental streak 1 and 2, have power to reproduce these diseases entirely for only 5 to 6 months, after which time it is assumed that the potato virus has lost its virulence, for the resulting symptoms are those of tobacco mosaic, or the mosaic of glasshouse streak only. After 12 months—no longer period has yet been tried—these symptoms appear regularly. . . . It is interesting to note that the virus of potato mosaic is less resistant than tobacco virus 1 or glasshouse streak to all three of these treatments."

In 1930 it was stated by Valleau and Johnson (13), "As the healthy potato virus does not appear to be able to withstand drying the possibility of a mixture with it '(true ringspot of tobacco)' seems to be eliminated."

As shown by Burnett and Jones (1), in a number of separate tests, the latent virus may be quite readily recovered from virus-infected potato, tomato, or tobacco leaf tissue after drying at room temperature for 10 to 46 days. Their records also show that the latent virus was recovered from virus-infected tomato and tobacco leaf tissue after being dried for periods of 77, 200, 233, and 286 days, respectively, and, moreover, that streak of tomato (latent plus tobacco mosaic) was recovered from dried tomato tissue that had been dried for periods up to and including 466 days. Likewise, the veinbanding virus was shown to remain active in dried plant tissue for at least 46 days.

In 1932 Johnson and Grant (8), working on the properties of different viruses, state, "With regard to the mottle form alone, the data for which are shown in parenthesis in table 7, there appears to be no evidence of any significant influence of the host species on the longevity *in vitro*." From the figures presented in that table it would appear that the spot necrosis viruses (veinbanding plus latent or rugose mosaic) are largely inactivated at the end of 20 days *in vitro*. The veinbanding virus (in the complex) is partly inactivated after two days with a more or less gradual diminution or a complete loss at the end of 20 days. A similar but decidedly slower inactivation is also noted for the latent virus.

MATERIALS AND METHODS

The experimental work was carried on in the greenhouse at the State College of Washington, Pullman, Washington, during the period from September, 1929, to March, 1933. Methods similar to those employed and reported in a previous article by Burnett and Jones (1) were followed throughout most of the experiments. A modified maceration method of inoculation as reported by Jones (9) was employed.

Juice from potato, tomato, and tobacco plants naturally infected or artificially inoculated with a single virus or combination of viruses was tested on tobacco or tomato plants to determine the presence of these viruses. The foliage from these infected plants was placed in open paper bags and allowed to dry in a section of the greenhouse under thermostatic control, the temperature generally ranging from 68°–75° F. Collections were made at various periods throughout the experiment and the dried inoculum was tested on tobacco or tomato plants at intervals of time in order to determine the longevity of the viruses in dried plant tissue. The John Baer variety of tomato and Connecticut Havana tobacco were used in these experiments. Because of the shortage of available space and the large number of series tested over a period of nearly 4 years, it was impossible to test all series at the same time. For this reason the various series were tested at varying intervals of time and then grouped and tabulated, as shown in the tables.

When a given inoculum failed to give positive symptoms on the test plants it was discarded and no further tests were made.

In some series only 2 plants were used as test plants. This was especially true when the inoculum had been dried but a short time. As the length of drying increased and the percentage of infection decreased in a given series, the test plants in the subsequent series were increased to 5, 10, or even 20. It is obvious that if the percentage of infection is low the recovery of the virus will be more likely if a greater number of test plants are used.

LONGEVITY OF THE LATENT VIRUS

Tests were made of the longevity of the latent virus of potato when dried in potato, tomato, or tobacco leaf tissue. The determinations were made by using the macerated leaf tissue after various periods of drying to make inoculations into young tobacco and tomato plants. The results are presented in table 1, with an interpretation of the figures offered in a footnote.

Using the fresh leaf tissue, a number of different sources were tested for each host: 62 series for potato; 27 for tomato; and 42 for tobacco. In the potato series 57 different lots inoculated on 229 tobacco plants gave 197 with symptoms, while 5 series tested on 2 plants each gave negative symptoms. Of the tomato series 20 were positive and 7 negative, and for tobacco 38 were positive and 4 negative. For leaf tissue dried 3 days or for longer periods, in general, a lesser number of sources or lots were used in a similar way for each of the hosts. If there was any doubt whatever regarding the symptoms on any series the results were recorded as negative. This may account for the negative results when fresh inoculum was used.

A further analysis of tables 1 and 2 also reveals the fact that, with the fresh inoculum, the percentage of series giving positive results is 72.2 to 91.9 per cent, whereas the percentage of test plants showing positive symptoms, in these series, varies from 82.4 to 86.4 per cent. In general, the longer the inoculum was dried the lower was the percentage of infection. This also accounts for the increase in the number of series giving negative results as the length of time of drying is increased. There is insufficient evidence from the data presented to state that the latent virus will retain its infectivity longer in one host than in another. At the end of 3 days the percentage of infection varied from 95 to 100 per cent.

When potato foliage that had been dried for 3 to 28 days was tested, as shown in table 2, 66.6 per cent of the series gave positive results. For the periods 29–50, 132 and 263 days, the recovery was 50, 1.7, and 100 per cent, respectively. These figures are not so erratic as they may seem when it is noted in table 1 that only one series of the 57 tested gave positive re-

TABLE 1.—*Results of the longevity tests of the latent virus of potato when dried in potato, tomato, or tobacco leaf tissue and reinoculated on tobacco and tomato plants*

No. of days dried	Recovery of the virus from dried foliage of					
	Potato		Tomato		Tobacco	
	Positive	Negative	Positive	Negative	Positive	Negative
None "Fresh inoculum"	57–229[1] / 197	5–10 / 0	20–140 / 121	7–35 / 0	38–154 / 127	4–20 / 0
3	8–40 / 40	4–20 / 0	12–60 / 57	.....	12–60 / 60	.....
10	8–40 / 28	4–20 / 0	10–50 / 31	2–10 / 0	11–55 / 43	2–10 / 0
11 to 13	4–20 / 6	2–10 / 0	4–20 / 9	2–10 / 0	19–175 / 91	5–55 / 0
17	8–40 / 22	4–20 / 0	10–50 / 35	2–10 / 0	10–50 / 31	4–20 / 0
20	.....	.....	.....	.....	10–100 / 29	11–110 / 0
28	4–20 / 17	2–10 / 0	4–20 / 5	2–10 / 0	3–15 / 4	3–15 / 0
29 to 50	8–40 / 18	8–40 / 0	11–55 / 24	7–45 / 0	10–50 / 31	6–30 / 0
72 to 175	1–10[2] / 1	56–560 / 0	.....	.....	2–20[3] / 4	13–130 / 0
250 to 325	1–10[4] / 2	.....	.....	2–40 / 0	1–20[5] / 6	10–140 / 0
326 to 775	.....	13–170 / 0	.....	35–540 / 0	.....	9–110 / 0

[1] First number of numerator represents total number of series or sources of inoculum; second number, the total number of plants inoculated; and the denominator represents the number of plants showing symptoms of the latent virus.

[2] Dried 132 days.

[3] Dried 72 days.

[4] Dried 263 days.

[5] Reported by Burnett and Jones (1)—dried 286 days.

sults for the period of 72 to 175 days, with a 10 per cent infection, and only one series tested gave positive results for the 263 day period with a 20 per cent infection. The percentage of plants infected, when inoculated with the potato foliage, varied from 100 for the 3 day period to 10 for the

263 day period, the latter being the longest period for which the latent virus was recovered from dried potato foliage. With a few exceptions there is a rather gradual reduction in the percentage of infection as the length of drying is increased.

In a similar manner, the tests with tomato foliage, as shown in tables 1 and 2, gave some variations in the percentage of series giving positive results and in the percentage of plants showing symptoms. Only 72.2 per cent of these series with the fresh inoculum showed recovery; 100 for the 3-day test and a fairly gradual diminution to 61.1 for the 29–50-day test.

TABLE 2.—*Positive recovery of the latent virus from dried material in per cents of positive series and positive plants. Based on results in table 1.*

No. of days dried	POTATO [1]		TOMATO		TOBACCO	
	Per cent series positive	Per cent plants positive	Per cent series positive	Per cent plants positive	Per cent series positive	Per cent plants positive
None "Fresh inoculum"	91.9	86	72.2	86.4	90.4	82.4
3	66.6	100	100	95	100	100
10	66.6	70	83.3	62	84.5	78.1
11 to 13	66.6	30	66.6	45	79.1	52
17	66.6	55	83.3	70	71.4	60.2
20	.....	.....	.....	.....	47.5	29
28	66.6	85	66.6	25	50	26.6
29 to 50	50	45	61.1	43.6	62.5	60.2
72 to 175	1.7 [2]	10	.....	.....	13.3	20
250 to 325	100 [3]	20	0	.....	9.09	30
326 to 775	0	.....	0	.....	0	.....

[1] The first column under each host represents the per cent of series giving positive results; the second column under each host represents the per cent of inoculated tobacco plants in the positive series showing symptoms of the latent virus.

[2] Dried 132 days.

[3] Dried 263 days.

There was some fluctuation in the number of plants showing positive symptoms, with a greater percentage of infection with the fresh inoculum and the material dried for 3 days; but, again, it is evident that the foliage that had been dried longer generally gave a lower percentage of recovery. The virus was not recovered from tomato foliage that had been dried more than 50 days.

When tobacco foliage was tested, as shown in tables 1 and 2, the fresh inoculum showed 90.4 per cent of the series positive, 100 per cent for the

3-day test, with a more definite and gradual decline to 9.09 per cent for the 286-day test. In the latter case when tobacco foliage had been dried 286 days, 6 plants of the 20 inoculated gave positive symptoms of the latent virus.

After the inoculum had been dried for 72 days or more, only 5 separate series, 2 with the potato foliage and 3 with tobacco foliage, gave positive results of recovery of the virus, and the infection varied from 10 to 30 per cent.

There was no consistent or significant difference in the longevity of the latent virus when secured from apparently healthy potatoes or from potatoes that showed definite symptoms of rugose mosaic, crinkle mosaic, leaf roll or other viroses. Definite data are not presented in the tables, but naturally infected rugose-mosaic potato plants (latent plus veinbanding), when tested on tobacco plants, reveal the fact that, in general, the latent virus withstands aging longer than does the veinbanding virus. Similar results were presented by Johnson and Grant (8) for the spot-necrosis viruses (rugose mosaic) when tested *in vitro*.

Whether virus-infected plant parts are dried rapidly or slowly may determine to a large extent how long a specific virus may be recovered from such infected host tissue. Definite figures are not presented, but in working with numerous collections, it appeared that the virus retained its virulence much longer in infected plant tissue when dried rapidly than when dried slowly or subjected to fluctuating air humidity or to alternate wetting and drying in the soil.

LONGEVITY OF THE VEINBANDING VIRUS

A corresponding series, similar to that presented for the latent virus, was conducted to determine the longevity of the veinbanding virus. Experimental results that show the recovery of the veinbanding virus from dried foliage of potato, tomato, and tobacco are presented in table 3. The tabulations are arranged according to the same plan as in table 1. The infection with fresh inoculum approaches nearly 100 per cent. There was little additional inactivation at the end of 3 days. At the end of 10 days there was a noticeable decrease in the percentage of recovery. This was likewise true for the 17-day test. The veinbanding virus was not recovered from tomato foliage that had been dried more than 17 days. It was recovered from dried potato and tobacco foliage at the end of 31 and 50 days, respectively, with approximately 70 per cent of the test plants showing positive symptoms for the 50-day period, but it was not recovered when the foliage had been dried for 100 days or more.

The source of the veinbanding virus did not appreciably influence its longevity in the dried foliage of the 3 hosts. The percentage of recovery

TABLE 3.—*Results of the longevity tests of the veinbanding virus when dried in potato, tomato, or tobacco leaf tissue and reinoculated on tobacco plants*

No. of days dried	Recovery of the virus from dried foliage of					
	Potato		Tomato		Tobacco	
	Positive	Negative	Positive	Negative	Positive	Negative
"Fresh inoculum"	19–38 [1] / 34	2–3 / 0	2–4 / 4	.....	48–85 / 81	2–10 / 0
1 to 2	.....	.....	.....	.....	2–10 / 9	.....
3	6–30 / 29	2–10 / 0	5–25 / 19	3–15 / 0	9–45 / 44	.....
4 to 8	.....	.....	.....	.....	5–25 / 15	.....
9	2–10 / 5	2–10 / 0	1–5 / 5	3–15 / 0	4–20 / 10	.....
10	6–30 / 22	3–15 / 0	2–10 / 7	7–35 / 0	6–30 / 18	4–25 / 0
11 to 13	.....	.....	.....	.....	3–15 / 7	4–40 / 0
14 to 16	.....	.....	.....	.....	.....	4–40 / 0
17	5–25 / 18	3–15 / 0	1–5 / 1	8–40 / 0	4–20 / 13	6–30 / 0
18 to 24	.....	.....	.....	.....	.....	17–175 / 0
31	3–15 / 5	6–30 / 0	..... /	8–40 / 0	1–5 / 2	7–35 / 0
50	4–40 / 28	2–10 / 0	.....	2–20 / 0	2–20 / 15	2–20 / 0
100 to 362	.....	56–730 / 0	.....	22–320 / 0	.....	14–160 / 0

[1] First number of numerator represents total number of series or sources of inoculum; second number, the total number of plants inoculated; and the denominator represents the number of plants showing positive symptoms of the veinbanding virus.

from the tomato foliage appears to be somewhat less than from the potato or tobacco foliage. The possibility of recovery from tomato appears to be somewhat lower, but this fact is not necessarily significant when the number of tests presented are taken into consideration. With a great number of tests this fact may or may not be borne out.

LONGEVITY OF THE LATENT VIRUS WHEN IN COMBINATION WITH TOBACCO MOSAIC (STREAK OF TOMATO)

Results of the longevity tests for the latent virus in combination with tobacco mosaic (streak of tomato), when dried at room temperature in potato, tomato, or tobacco leaf tissue and reinoculated in tomato plants are presented in table 4. Tests up to and including 466 days, as reported by Burnett and Jones (1), are not included in this report.

The latent virus used in these tests was originally secured from potato plants naturally infected with one of the following diseases; crinkle mosaic, latent (apparently healthy potatoes), leaf roll, rugose mosaic, and spindle tuber. Potato seedlings as well as tomato and tobacco plants were inoculated with tobacco mosaic and the latent virus from one of the above sources. After the viruses had been given time to become systemic in the plants or had given definite symptoms, the foliage was removed and dried as reported under "Materials and Methods." This foliage, after being dried for variable periods of time, was retested on tomato plants as indicated in table 4. The tabulated results are not intended to show the percentage of tobacco mosaic recovered but to demonstrate the recovery of the latent virus. In nearly all of the tests, however, the recovery of tobacco mosaic from tomato and tobacco foliage was 100 per cent. When the inoculated potato seedling foliage was tested the percentage of recovery of tobacco mosaic was very low. The entire foliage of the inoculated potato seedlings was collected and used as a source of inoculum. Since the potato is often resistant to systemic infection with tobacco mosaic, it is possible that when tobacco mosaic was recovered in the test plants it was obtained from the point of local infection of the inoculated leaf.

The tabulations are arranged according to the same plan as in table 1. It will be noted that 2 plants of the 20 inoculated, for the period of 325 to 425 days, with inoculum from the potato foliage gave positive results. This is the longest time that the latent virus was found to remain infective when dried in potato foliage. In a similar manner when the combination of the latent virus and tobacco mosaic was dried in tobacco foliage it retained the infective agent of the latent virus for 618 days. This gave in 2 separate series 20 per cent infection (the production of streak on tomato), which is represented by 8 out of 40 plants showing positive symptoms. When foliage from tomato plants, which exhibited true streak (latent plus tobacco mosaic), was

TABLE 4.—*Results of the longevity tests of the latent virus, in combination with tobacco mosaic (streak of tomato) when dried at room temperature in potato, tomato, or tobacco leaf tissue and reinoculated on tomato plants*

No. of days dried[1]	Dried in following host	Recovery of the latent virus when the following virous diseases of potato were used as the original sources of inoculum									
		Crinkle mosaic		Latent		Leaf roll		Rugose		Spindle tuber	
		Pos.[2]	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.
325 to 425	Potato seedling	.....	1–10 [3] / 0	.....	2–20 / 0	1–20 [4] / 2	.....	.....	9–130 / 0	.....	.....
	Tomato	4–70 / 7	13–210 / 0	2–40 / 8	6–70 / 0	1–20 / 1	2–30 / 0	4–80 / 12	14–250 / 0	1–20 / 1	4–70 / 0
	Tobacco	.....	1–10 / 0	.....	.....	1–20 / 2	.....	.....	3–50 / 0	.....	.....
550 to 750	Potato seedling	.....	.....	.....	1–10 / 0	.....	1–20 / 0	.....	1–20 / 0	.....	.....
	Tomato	3–60 / 10	2–40 / 0	.....	.....	1–20 [5] / 1	1–20 / 0	.....	.....	.....	.....
	Tobacco	.....	1–20 / 0	2–40 [5] / 8	.....	.....	.....	.....	1–20 / 0	.....	.....
758	Tomato	1–20 [6] / 3	2–40 / 0	.....	.....	.....	.....	.....	.....	.....	.....
826	Tomato	1–20 [6] / 2	.....	.....	.....	.....	.....	.....	.....	.....	.....
1202	Tomato	1–20 [6] / 3	.....	.....	.....	.....	.....	.....	.....	.....	.....
1251	Tomato	1–20 [6] / 2	.....	.....	.....	.....	.....	.....	.....	.....	.....

[1] Longevity tests of the latent virus (streak of tomato) up to and including 466 days previously reported by Burnett and Jones (1).

[2] Pos. = symptoms evident; Neg. = no symptoms.

[3] First number of numerator represents the total number of series or sources of inoculum; second number the total number of plants inoculated; and the denominator represents the number of plants affected with streak. Nearly 100 per cent of plants gave positive symptoms of tobacco mosaic, except inoculum from potato foliage, which in general gave negative results.

dried and retested on tomato, the latent virus was sufficiently virulent to produce 10 to 15 per cent infection after being dried for 1251 days, or approximately $3\frac{1}{2}$ years. This particular streak inoculum was collected on July 30, 1929, by Dr. L. K. Jones from tomato plants that had previously been inoculated with the latent virus from potatoes showing crinkle mosaic, and with tobacco mosaic. This inoculum was dried very rapidly when collected because of the prevalent low humidity and the high temperature. At that time it was not uncommon to have the temperature reach 95° to 100° F. during the daytime. This inoculum was still infective when the last test was made on January 2, 1933.

It has been noted throughout these experiments that when the inoculum is dried more rapidly there is a tendency for the latent virus to retain its virulence for a longer time. A similar result was obtained with tobacco mosaic by Johnson and Ogden (7) who state that, "Moist and well aerated soils favor the inactivation of the virus as compared with dry, compact or waterlogged soils." With fresh streak inoculum it is not uncommon to obtain 100 per cent infection of streak on tomato plants. As the period of time of drying is increased the percentage of recovery is reduced. With inoculum that had been dried for 6 months or more, where the recovery of the virus was secured, the results showed from 5 to 20 per cent infection. If only a small number of plants are inoculated, there is much less probability of recovering the latent virus if the percentage of infection is low.

It is interesting to note (Tables 1 and 4) that when the latent virus was dried alone in plant tissue it did not survive so long as when dried in the presence of tobacco mosaic (streak). By referring to table 1 it will be noted that the longest period for which the latent virus was recovered from dry plant tissue when used alone was 286 days. When the latent virus (as secured from potatoes with crinkle mosaic) was combined with tobacco mosaic in tomatoes to produce streak it was found infective after being dried for a period of 1251 days, or approximately $3\frac{1}{2}$ years (Table 4). Other data from table 4 also show that the latent virus when combined with tobacco mosaic retained its infectivity longer than 286 days, as pointed out for the latent virus when dried alone. It is not clear just what influence the tobacco mosaic has on the longevity of the latent virus when the 2 viruses are dried in combination in plant tissue. From the results as indicated in these experiments, it would appear that the latent virus remains virulent much longer when found in combination with tobacco mosaic than when dried alone in leaf tissue. The 2 tomato plants that gave positive streak symptoms after having been inoculated with the streak inoculum that had been dried for a period of 1251 days were further tested on *Datura stramonium* to determine definitely the presence of the latent virus. The 10 Datura plants, thus inoculated, gave the local lesions on the inoculated leaves characteristic of tobacco

[3] First number of numerator represents the total number of series or sources of inoculum; second number the total number of plants inoculated; and the denominator represents the number of plants affected with streak. Nearly 100 per cent of plants gave positive symptoms of tobacco mosaic, except inoculum from potato foliage which in general gave negative results.

mosaic, while on the younger foliage only the mottle characteristic of the latent virus appeared. This gave further evidence that the latent virus was present and infective in the streak inoculum that had been dried for 1251 days.

A further test was made that demonstrates the presence of the latent virus in the above streak material. Five healthy potato plants of the Early Rose variety, which had been tested and found to be free of the latent as well as other viruses, were inoculated with the streak inoculum. Following a 2-week period of incubation, the foliage from each of the 5 potato plants was tested on tomato plants to which fresh tobacco mosaic was added. All of the tomato plants thus inoculated gave a severe type of streak.

SUMMARY

The latent virus when dried alone in plant tissue was found infective after being dried for 286 days in tobacco, 263 days in potato, but for not over 50 days in tomato. These results were obtained from inoculations involving 510 series of dried inoculum on 3743 tobacco and tomato plants.

The veinbanding virus, when dried alone in plant tissue, was found infective after being dried for 50 days in potato and tobacco but for only 17 days in tomato. These results were obtained from inoculations in which 329 series of dried inoculum on 2355 tobacco plants were used.

The latent virus, when dried in foliage in combination with tobacco mosaic (streak of tomato), remained infective longer than when dried alone. It (in the combination) was recovered from potato, tomato, and tobacco foliage that had been dried for periods of 352, 1251, and 618 days, respectively. These results were obtained from inoculations using 86 series of dried inoculum on 1510 tomato plants.

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FREQUENT ASSOCIATION OF PHYTOMONAS MELOPHTHORA, WITH VARIOUS STAGES IN THE LIFE CYCLE OF THE APPLE MAGGOT, RHAGOLETIS POMONELLA[1]

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INTRODUCTION

Phytomonas melophthora A. and R., which causes rot of ripe apples, has been studied in relation to various stages in the life cycle of the apple maggot, *Rhagoletis pomonella* Walsh. This work was undertaken because of the various indications (reviewed by Allen and Riker (2)), of a frequent relation between the larvae of this insect and the rot organism. It seemed desirable to determine, if possible, how the larvae and the bacteria became associated and the frequency of this association throughout the life cycle of the insect.

The economic importance of the combination of *Phytomonas melophthora* with the apple maggot is manifest both in the orchard and in storage. Under natural conditions in the orchard the dissemination of the bacteria and the mode of their entry into the fruit are apparently dependent upon the insect. Under storage conditions subsequent development of rot in the fruit seems associated with the presence of maggot infestation. Consequently, further study of this relation appeared desirable.

The problem has been studied primarily by means of certain bacteriological methods applied to various stages of the insects and to their immediate environment. This type of study has already shown its value in the excellent work by Leach (6) and Johnson (4) on the relation of certain insects to the spread of soft-rot bacteria. The technic employed by the writers is reviewed briefly.

MATERIALS AND METHODS

The materials used in these studies included (1) the various available stages in the life cycle of the insect and (2) the accompanying decay in apple tissue, as listed in table 1. Infested fruits were obtained in 1931 and 1932 from Gays Mills, Madison, Sparta, and Winneconne, Wisconsin. Adult flies and the fruits that contained both eggs and the ovipositor punctures were collected at Gays Mills. Several varieties of maggot-infested apples were used in the studies: viz., Dudley, McMahon, Snow, Wealthy and Yellow Transparent.

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. These studies have been made in cooperation between the Departments of Economic Entomology and Plant Pathology.

Isolations from apple tissue were made in accordance with the usual laboratory technique as employed by Allen and Riker (2), except as noted.

Isolation studies from the various stages of the insect involved variations in procedure. Adult flies, eggs, egg-shells, and larvae were placed in broth and removed after 20 minutes. The broth was incubated 48 hours at room temperature before dilution plates were poured. Suspensions from the broth were transferred into healthy apples. When bacterial decay developed in the apple tissue, poured-plate isolations were made on nutrient glucose agar. Flies were killed with chloroform or ether before crushing in tubes of nutrient glucose broth.

Methods for treatment of flies, eggs, and larvae with surface disinfectants were as follows: The material was placed in 70 per cent alcohol for 3 minutes to remove air, washed in sterile water and transferred to bichloride of mercury, 1 to 1000. After 5 minutes it was washed in sterile water and placed in a tube of broth for 20 minutes. Then it was transferred to another tube of broth and crushed. The bacteria on the surface, reached by this method, were certainly killed, but the possibility remains that those embedded in slowly soluble material upon the surface may have escaped injury. The natural openings make it difficult to differentiate between surface and partial internal sterilization. However, in the data that are presented later, the interpretation is made that, when the tubes of broth in which the material remained for 20 minutes remained sterile, any bacteria subsequently recovered came from inside. The development of growth in a check broth tube was interpreted to mean that microorganisms occurred on the surface of the material and no further isolations were attempted. When growth appeared in the tube where the insect was crushed, inoculations with the broth were made into apples and reisolations were made as previously described (2).

Eggs, which were nontreated and incubated to obtain developing larvae, were removed from apple tissue and transferred aseptically to dark moistened blotting paper within small sterilized vials. These vials were kept slightly above room temperature. As larvae hatched, they were transferred aseptically to needle pricks in surface-sterilized fruits. As decay developed in the apples, isolation studies were made of the rot that developed adjacent to the larval burrows. Certain difficulties of technique remain to be overcome with eggs treated with a surface disinfectant, for only 2 out of 25 trials were successful. In these 2 successful attempts, the larvae from treated eggs died a couple of days after being placed in apple tissue. No rot developed in the surrounding fruit tissue.

Several methods were tried before successful isolation of the organism was obtained from the pupal stage. The following method gave the most satisfactory result. Puparia were removed from soil and placed in 5 cc.

of physiological salt solution for 20 minutes with frequent agitation. This salt solution was diluted approximately 1 to 6, 1 to 35, and 1 to 200. One cc. from each dilution was placed into each of 3 Petri dishes with glucose yeast-infusion mineral-salt agar. This medium was similar to that employed by Wright (7). Its composition was glucose, 10 grams; magnesium sulphate ($MgSO_4 \cdot 7\ H_2O$), 0.2 grams; sodium chloride (NaCl), 0.2 grams; dipotassium phosphate ($K_2HPO_4 \cdot 3\ H_2O$), 0.1 gram; calcium chloride ($CaCl_2$), 0.1 gram; 10 per cent yeast infusion, 100 cc. and distilled water 900 cc. The reaction was adjusted to pH 6.8.

Puparia were also treated in disinfectant as described earlier, except that they were previously washed 3 times in tubes of sterile salt solution. Instead of 3 dilutions as used earlier, 8 successive dilutions were made to approximately 1 to 1,000,000. Three plates were poured from each dilution, making a total of 24 plates from each puparium. This relatively large amount of work was performed because it increased the chances for success.

The pathogenicity of the cultures isolated was determined in each case on one of several varieties that included Delicious, McIntosh, Wagner, and Yellow Transparent.

The results secured with these methods are given briefly in the following pages.

RESULTS

Phytomonas melophthora, the cause of bacterial storage rot of apples in Wisconsin, has been found associated with all stages examined in the life cycle of the insect, *Rhagoletis pomonella.* The life cycle of this insect, with its accompanying plant pathogen, has been followed in material from the orchards at Gays Mills. Certain parts of the cycle were studied with material from other sections of the State. The results of isolation studies from the various stages of the insect and from corresponding decay in apples are reported in detail in the following pages and are summarized in table 1. The same sequence of the work on different forms of the insect and associated apple tissue is followed both in the text and in this table.

ADULT FLIES

The adult flies used in these experiments were commonly found harboring *Phytomonos melophthora* in such quantities that they were readily isolated by methods outlined above. The reasons for suspecting the adult fly as a vector in the production of this disease were (1) the rot that sometimes followed the wound produced during oviposition in relatively ripe fruit (Fig. 1, C), and (2) the association of the apple maggot with rot, as considered earlier (2).

The flies examined were secured, 13 from the orchard and 12 from a series of emergence cages previously placed over piles of infested fruit.

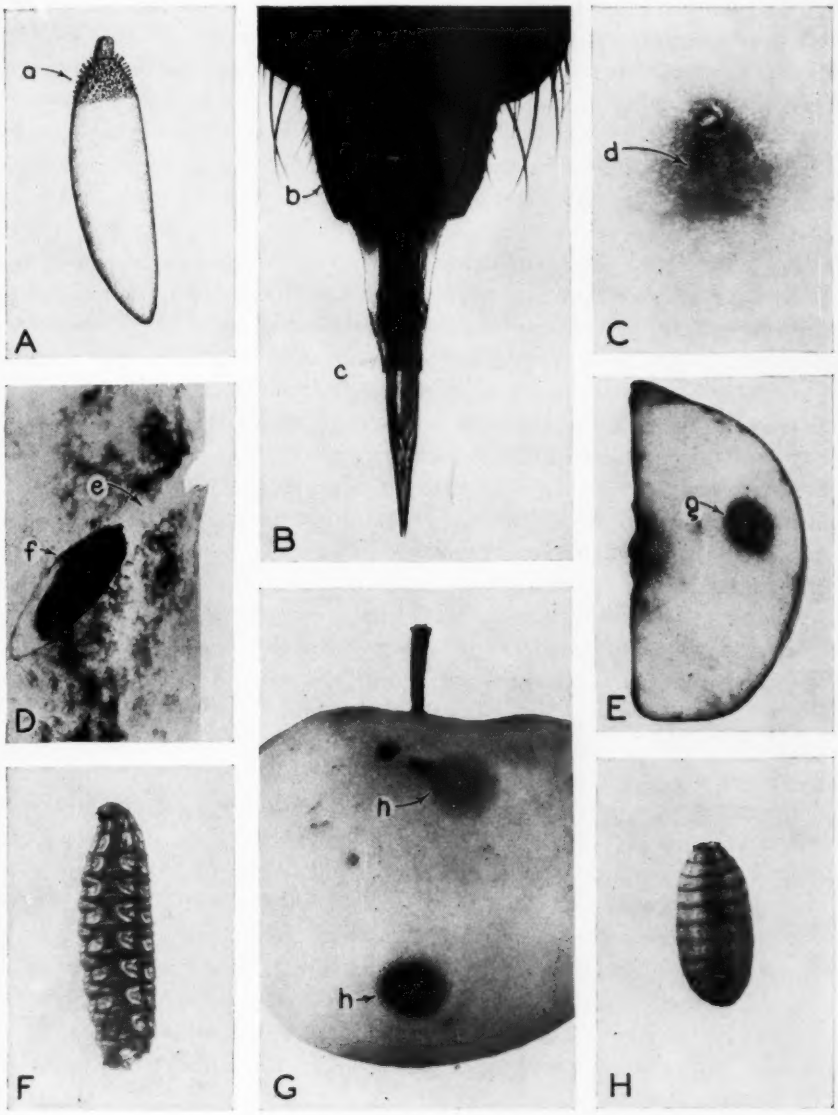

FIG. 1. A. Egg of the apple maggot and reticulation, *a*, at posterior end. ×51. B. The posterior of a female fly, showing the end of the abdomen, *b*, and the partially extended ovipositor, c. ×23. C. Egg puncture viewed from the surface of a ripe apple. Decayed tissue, *d*, appears about the puncture. ×15. D. The egg, *f*, at normal position in apple tissue, showing ovipositor or egg puncture, *e*. ×31. E. Rot, *g*, about larval burrow in apple. ×1½. F. Mature larva. ×6. G. Rot, *h*, about larval exit holes in apple. ×1½. H. Puparium. ×4.

Two broods of flies, some of which were obtained from 2-year-old puparia, were involved in the collection as explained by Allen and Fluke (1). The age of the flies collected in the orchard was uncertain, but only 1-day-old flies were used from the emergence cages.

Isolations were made to determine whether the flies were actually carrying the bacteria. The flies were subjected to the fumes of either chloroform or ether before the isolations. Both male and female flies were used for dilution plates either with or without surface treatment with a disinfectant as follows: Eight of 10 trials with nontreated female flies were positive for *Phytomonas melophthora*. Three trials with treated females were all positive. Four of 6 trials with nontreated male flies were positive. All of 6 trials with treated males were positive. These results are summarized in table 1. The larger percentage of positive results from the treated flies was perhaps the result of freedom from troublesome contaminations. Since about half of the males and females were only a day old and were from emergence cages it is suggested that they carried the bacteria either from the puparia or perhaps from the surrounding material. This question is considered further in the next paragraph and in connection with studies of the puparia. From these studies it appears that *Phytomonas melophthora* may be carried both internally and externally by the mature fly in quantities sufficiently large to account for the observed dissemination of the bacteria in Wisconsin orchards. This raises the question concerning how the association occurred.

Soil isolations were attempted in an effort to learn whether the flies became contaminated as they emerged. Four soil samples obtained within emergence cages were examined. Each soil sample was collected from the surface 2 inches in a sterile glass vial, mixed and divided into approximately 2 equal portions. One portion was plated in nutrient dextrose agar in triplicate from 6 dilutions that ranged up to 1 in 2,000,000. Part of the other portion of each sample was introduced into apple according to the method of Ark (3) with the exception that ripe Yellow Transparent apples were substituted for green pears. The negative results secured, perhaps because of inadequate technique, are not interpreted to mean that the soil does not harbor *Phytomonas melophthora*, but that, under the conditions of the above experiment, this organism was probably not present in the soil in relatively large quantities. Since more than three-fourths of the flies emerging from this soil carried the pathogenic bacterium, it appears more probable that the insects carried the bacteria from the puparia. How the flies transmitted the bacteria to the eggs was next considered.

Ovipositor Punctures

The ovipositor puncture was studied to determine the frequency of its inoculation. Five different varieties of apples were collected from 4

sources in the State, as mentioned earlier. The wounds made during oviposition, (Fig. 1, C) were located with binoculars. With sterile instruments the tissue of the fruit was cut away exposing the ovipositor puncture, *e*, and egg, *f* (Fig. 1, D). Isolations were made from the wound according to methods previously discussed. Results of isolations from 22 ovipositor punctures revealed that 10 of these contained *Phytomonas melophthora*. In connection with these results one should recall that relatively green apple tissue is not a favorable medium for the rot bacteria (2). These results suggest that bacteria may be transferred by means of the ovipositor (Fig. 1, B) and that, when conditions are favorable, rot may develop in the fruit following injuries made by the fly. Only a small percentage of the punctures were found devoid of eggs or egg-shells. The rate at which rot developed about the wound appeared to be influenced by the maturity of the fruit.

The inoculation of the ovipositor puncture might be accounted for by the habit of the adult fly. The ovipositor is extended and forced through the epidermis by several up and down movements. Any bacteria present on the ovipositor may therefore be smeared over the surrounding apple tissue. Following egg deposition, the ovipositor is generally cleaned by the rear legs of the insect before it is withdrawn into the abdomen. This cleaning habit also is performed in the same manner following defecation and can perhaps explain the distribution of rot bacteria over the ovipositor. Knight (5) has suggested that 2 pathogenic fungi were carried on the ovipositor of the apple maggot.

Eggs

Eggs of the insect (Fig. 1, A and D, *f*) were deposited a short distance under the epidermis of the apple fruit. Fifteen eggs were dissected from the apple parenchyma and isolations made from surface washings, according to the above-described technic. The results of these isolations showed that eight of the eggs were contaminated with *Phytomonas melophthora*. The projections on the proximal end of the egg (Fig. 1, A, *a*) apparently serve both to prevent any reverse movement of the egg and perhaps to harbor bacteria.

Eggs from captive flies were obtained by placing pairs of flies in a glass rearing cage together with a ripe apple, and a little yeast-water honey mixture. After the adult had been observed to deposit the egg, the fruit was removed from the cage, the egg dissected from the host tissue and, examined as earlier described. Three eggs were successfully extracted and isolations made from their surfaces without injury to the egg. In each attempt *Phytomonas melophthora* was recovered. In addition, 2 others were successfully obtained and treated with disinfectants without killing the contained embryos. The larvae were subsequently hatched and were found

to be free from *Phytomonas melophthora* as described later. While the number is too small for conclusions, this evidence suggests that the bacteria do not occur inside the egg. The number of eggs employed is small because of the difficulty already mentioned that was experienced in removing them from the apple tissue and in handling them without injury.

Egg Shells

Egg shells found in apple tissue were treated in the same manner as the eggs. Larvae had emerged from the eggs after 4 to 6 days. Results of isolations from 24 egg shells showed that one-third of them carried the rot bacteria. It appears from the evidence available that as the larva emerges from its shell, it may frequently make contact with the apple-rot bacteria.

Larvae from Orchard

Young larvae were collected in apples taken from the orchard. Isolations made from 13 such larvae, not treated with surface disinfectants, showed that 3 of them were carrying the bacteria. It is probable that when the bacteria were present they were unable to increase in number much, if any, because of the relatively unfavorable condition in the green fruit. Similar larvae treated with surface disinfectants gave the same results from the same number of trials as those not treated. This indicated that the bacteria were present inside as well as on the surface of the larvae. Since the early life of the larvae is usually spent in relatively green fruit, decay rarely develops about the wounds made during oviposition or about the egg. The larvae hatch, but development is retarded until the fruit reaches a relatively mature condition. The number of bacteria available for isolation from such larvae as have hatched within 2 or 3 days, therefore, is quite small. If the parasitized fruit is removed from the tree while still in a "green" condition, further progress of both the larvae and rot seems to be dependent on the physiological processes of ripening and can be hastened or retarded by manipulation of the storage conditions. Larvae developed rapidly in ripe stored fruit usually amid conspicuous infection of the host parenchyma (Fig. 1, E, *g*). The above observations correspond to those following artificial inoculations on fruits in various stages of maturity (2).

Mature larvae (Fig. 1, F) from fallen fruit in the orchard were examined. Isolations from 13 of this group revealed that all contained *Phytomonas melophthora*. Thirteen similar larvae, which were treated with disinfectants, also revealed that all carried the plant pathogen. These trials showed that the mature larvae of this insect carried the rot-producing pathogen in relatively large numbers. While it was noted that the larvae apparently preferred the decayed portion of the fruit, no investigations

were undertaken on the relation of the products of decay to the nutrition of the insect.

Larvae from captive flies were obtained from the eggs laid in insect cages. Nontreated larvae from this source were allowed to develop normally in the apple tissue until they neared maturity. Six of them were then crushed in broth. In each trial *Phytomonas melophthora* was recovered from the larvae in large quantities. Larvae from nontreated eggs of captive flies also were hatched on sterile blotting paper. The larvae were placed in broth for 20 minutes and then transferred to sterile apple tissue. Rot was produced in each of these trials and the bacteria were recovered from both the apple tissue and the tubes of broth. These larvae pupated in the usual manner. Larvae from treated eggs of captive flies were hatched as above and placed in broth for 20 minutes and then into healthy apple tissue. No cloudiness appeared in the broth after 2 weeks' incubation and the larvae were assumed to be free from bacteria. After 3 days both of the insects used in this experiment had burrowed in the apple tissue for a few centimeters and had died. There was no apparent increase in size of the larvae. No signs of decay were detected in the apple tissue.

The rot that developed about the larvae from captive flies also showed the presence of the typical rot bacteria in each of 3 trials. No difference was observed in the character of the rot observed in the insect cages from that found in the orchard.

Larval Burrows

Larval burrows (Fig. 1, E, *g*) in a variety of stages of decay and development were tested for the presence of *Phytomonas melophthora* during the course of these studies. Nineteen attempted isolations showed that 12 of them were infected with the bacteria. A greater portion could have been obtained had the writers rejected the burrows made by very young larvae in unripe apple tissue.

Exit Holes

Exit holes in the fruit were commonly observed to show progressive decay in nature (Fig. 1, G, *h*). At this period the larvae had matured and worked out of the fruit to pupate in the soil. Spread of the rot about the exit holes progressed until the whole apple was decayed by *Phytomonas melophthora* or until other bacteria or fungi entered and completed this process in advance. Twenty-three apples were collected showing various stages of rot developing about exit holes. Positive cultures of the bacteria were secured from 11 of these fruits. Although all the exit holes showed decay, considerable difficulty was experienced in a number of cases owing to other bacteria and fungi. An unidentified fungus resembling Mucor

was particularly troublesome and was probably responsible for much of the failure to recover the apple-rot bacteria. The above experiments indicate that the larvae commonly enter the pupal stage carrying a considerable quantity of the plant pathogen.

Puparia

Puparia (Fig. 1, H) were collected and stored in sand in the ice box for approximately 7 months. The purpose of storage was to try to determine

TABLE 1.—*Summary of isolation studies made on the association of apple-rot bacteria with different stages in the life cycle of the apple maggot and on the decay of apple tissue*

Source of isolation	Specimens employed	Specimens yielding pathogenic cultures	Isolations successful
	No.	*No.*	*Per cent*
Adult flies treated[a]	9	9	100
" " nontreated	16	12	75
Ovipositor puncture	22	10	45
Surface of eggs	15	8	53
Eggs from captive flies			
Treated[a]	2	0	0
Nontreated	3	3	100
Egg shells	24	8	33
Larvae from orchard			
Young larvae treated[a]	13	3	23
" " nontreated	13	3	23
Mature larvae treated[a]	13	13	100
" " nontreated	13	13	100
Larvae from captive flies			
Nontreated	6	6	100
From nontreated eggs[b]	3	3	100
From treated eggs[ab]	2	0	0
Rot about larvae of captive flies	3	3	100
Larval burrows	19	12	63
Exit holes in fruit	23	11[c]	48
Puparial surface washings	25	16	64
Puparia treated[a]	20	0	0

[a] The treatment was with a disinfectant, as described in the text.

[b] The eggs were removed from the apples and hatched in vials.

[c] The low number of positive results was probably because of secondary organisms, as explained in the text.

if time had a bearing on mode of overwintering of the bacteria. Twenty-five healthy puparia were chosen from the group and isolations made from surface washings. Ordinary methods of isolation failed in studies of the puparia, so certain modifications were employed as described earlier. Sixty-four per cent of these puparia were found to carry *Phytomonas melophthora* on that portion of the puparia that can be wet by water. These results indicate that overwintering in the field is likely to occur on the surface of the puparia.

Puparia, treated with disinfectants before crushing, gave no evidence of internal transmission of *Phytomonas melophthora.* Twenty puparia were used in these attempts, all of which gave negative results. Considerable difficulty was experienced with other bacteria and fungi that appeared in large numbers on the dilution plates. Cultures were secured from almost all of the various colonies appearing on these plates, and inoculations made into apple fruits gave negative results. The fact presented earlier that 1-day-old flies often carried the rot bacteria suggests that these bacteria were associated with the fly in the pupal stage. In this and earlier isolation studies it should be borne in mind that the rot bacteria, when present, might easily be missed, especially in badly contaminated material. The results of the above experiments suggest that *Phytomonas melophthora* may overwinter more abundantly on the surface of the puparia than inside.

SUMMARY

Phytomonas melophthora, the apple-rot bacterium, has been studied in relation to the various stages in the life cycle of *Rhagoletis pomonella,* the apple maggot. The bacteria have been found commonly associated with both male and female adult flies, eggs, larvae, and puparia. They have also been found in the ovipositor punctures, larval burrows, and exit holes in apple-fruit tissue. The bacteria were recovered from adult flies, and larvae, following treatment with surface disinfectants.

This work, based on 244 isolations, 54 per cent of which were positive, indicates that these apple-rot bacteria may be frequently associated with various stages in the life-cycle of the apple maggot.

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STUDIES ON ALFALFA MOSAIC[1]

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A preliminary report on the occurrence of a transmissible virosis of the mosaic type affecting alfalfa (*Medicago sativa* L.) in California has been made previously.[2] As far as the writer has been able to learn, the existence of such a disease was not definitely proved prior to that time, although a few reports, evidently based on field observations, had been made. Miss J. I. Wood, of the Division of Mycology and Disease Survey, of the United States Department of Agriculture, in a letter to the writer, states that a mosaic of alfalfa has been reported from California, Montana, New York, Tennessee, Utah, and Washington. Dickson[3] reports that in one instance there appeared to be a successful transfer of mosaic from *Trifolium pratense* L. to *Medicago sativa* by means of aphids.

Elliott[4] attempted to infect alfalfa with the mosaics from sweet and red clovers but without success. Kunkel[5] lists alfalfa among the plants susceptible to a mosaic. F. R. Jones found what apparently is the same disease in Wisconsin and sent affected plants to the writer for study. While making an alfalfa-disease survey trip in 1928, what is thought to be the same disease was seen in a field near Twin Falls, Idaho. The disease also was found in Arizona in 1931. It seems probable that a careful survey would show the mosaic to be pretty generally distributed in many parts of the country. The purpose of this paper is to give a detailed description of the symptoms of the alfalfa mosaic previously reported[2] and to present experimental data to support the contention that the disease can be transmitted by aphids.

SYMPTOMS

The first evidence of the disease in a leaf is the appearance of one or more small, more or less circular, greenish yellow spots. These areas frequently consist of a yellowish band of tissue, ½ to 1 mm. in width, surrounding an island of apparently normal color, ½ to 2 mm. in diameter. In some cases

[1] Cooperative investigations between the California Agricultural Experiment Station and the Division of Forage Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture.

[2] Weimer, J. L. Alfalfa mosaic. Phytopath. 21: 122–123. 1931.

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FIG. 1. A. Alfalfa leaves showing characteristic mosaic lesions. B. Branches of an alfalfa plant sent to the writer by F. R. Jones from Madison, Wisc. The spots on these leaves are smaller and the crinkling is somewhat more pronounced than is typical of the mosaic described and illustrated elsewhere in this article. This plant is thought to have been affected with a different mosaic. Both about $\times \frac{2}{3}$.

there are two or more concentric rings of green surrounding narrow bands of chlorotic tissue.

The chlorosis gradually spreads until eventually the rings are more or less obscured. There may be only one spot on a leaf, but more frequently there are several. These spots often coalesce, involving considerable areas of the leaf (Fig. 1, A). The affected tissue is greenish yellow at first, but gradually becomes lighter, as more chlorophyll is broken down, until it is distinctly yellow, and in extreme cases, especially where the leaf remains on the plant for a long time, almost white in color. The chlorosis may spread in all directions from the central ring so that a considerable amount of the tissue may eventually become involved. Although the rings may lie between or on the veins, the yellow color characteristically runs parallel to the veins and frequently involves them. Besides being chlorotic, affected leaves may be reduced to 1/3 their normal size, crinkled, and more or less deformed (Fig. 2), although this happens only in severe cases, usually in the spring before the first cutting when the disease is most conspicuous. In some leaves there is little evidence of ring formation, although there is considerable mottling. Severely affected leaf tissue often is considerably thickened and brittle, breaking easily when bent. There appears to be some dwarfing of the affected stems when the disease is severe, but, for the most part, there is no visible effect on the growth of the plant. No necrotic lesions have ever been seen on the stems. Only the lower leaves of a plant or all of them, including those not fully expanded, may be affected (Figs. 2 and 3). Diseased spots do not fall out, but remain intact throughout the life of the leaf.

The disease does not cause premature defoliation.

METHODS

At first several of the methods commonly used for transmitting different viroses were tried. Affected leaves were ground in a mortar, the juice obtained was placed on the young leaves of growing plants, and needle pricks were made through the juice into the tissue beneath. Likewise, leaves were crushed between the thumb and forefinger and then healthy leaves were rubbed, but only negative results were obtained. Several other mechanical methods were tried but with negative results.

A species of leafhopper, believed to be *Empoasca fabae* Harris, and one of thrips, probably *Euthrips occidentalis* Perg., both of which are common on alfalfa in southern California, although not positively identified, were tested as possible vectors, with negative results. Several experiments were then conducted in which aphids, *Illinoia pisi* Kalt., were used as vectors. These will be discussed in some detail.

The cages used in these experiments were similar to those commonly employed in insect transmission work. They consisted of a wooden frame, 9½

FIG. 2. Stem of an alfalfa plant all of whose leaves show mosaic symptoms. Note crinkling in some of the lower leaves. About × 1.

FIG. 3. Stem of alfalfa plant with lower leaves showing numerous mosaic lesions and those at the top few or none. About × 1.

by 9½ by 16 in. in dimensions, covered on 3 sides with cheese cloth (45 meshes to the inch). In the 4th side, or front, window glass was inserted. The 2 ends were of ¾-in. board, in the center of which were cut circular openings 6 in. in diameter. The sides and the hole in the top were covered with cloth, while the other end rested on the pot containing the plants. A thick layer of cotton, placed about the plants, extended over the edge of the pots to prevent the entrance of insects between the base of the cage and the pot.

Unless otherwise stated, the aphids (25 to 75) were transferred from the breeding cages, where they were feeding on plants affected with mosaic, to the healthy plants, likewise growing under cages, by sucking them into a glass tube thrust through a hole in the cloth side and then blowing them into the cage enclosing the healthy plants. The opening in the cloth was kept tightly plugged with cotton when not in use. In Experiment 454 the aphids were shaken from twigs, taken from breeding cages, directly upon the healthy plants, and in Experiment 458 the same procedure was followed except that the aphids were first caught upon paper and then transferred to the plants. In both cases care was exercised to exclude other insects that might possibly have been in the breeding cage. The aphids were allowed to feed on the inoculated plants for from 48 to 72 hours and then were killed by spraying the plants thoroughly with nicotine and soap. The cages used did not exclude insects as small as thrips, either from inoculated or the control plants, but, since the control plants never developed mosaic, the presence of these insects was not considered important. The aphids in the breeding cages were collected originally from alfalfa growing in the field. Those in the original collection were identified by Ralph H. Smith, Entomologist of the University of California, as the pea aphid (*Illinoia pisi* Kalt.) and subsequent collections were assumed to be the same. Alfalfa plants of the Chilean variety growing in mountain subsoil in 8-in. pots were used throughout. For the most part, the seedlings were inoculated when about 2 months old. Controls, consisting of plants of the same lot held under cages, but without the addition of aphids, were run in all the experiments and in no case did any of these become infected.

No mosaic symptoms ever developed as the result of the feeding of aphids on the plants in those experiments in which negative results were obtained. Likewise, alfalfa suffering from heavy aphid infestation has been observed in many localities without mosaic symptoms appearing. Once a plant is infected with mosaic, the symptoms may reappear after each cutting, even though the plant be kept entirely free from insects. This recurrence of symptoms would not occur if the trouble were due directly to the insects feeding upon them. These facts convinced the writer that aphids were not themselves responsible for these mosaic symptoms, but only served as vectors, hence controls in which noninfective aphids were placed on healthy plants were deemed unnecessary.

EXPERIMENTAL DATA

The data obtained in 6 experiments are given in table 1. The dates on which the aphids were placed upon the plants and those on which the first symptoms were observed are shown in the second and third columns. The difference between these dates in each experiment, usually 7 to 14 days, denotes the approximate incubation period. There appears to have been an unusually long incubation period in experiment 421. No explanation for this can be given. It may be stated, however, that the seedlings in this experiment were younger when inoculated than those commonly used, having only their first true leaves. The temperature for some time preceding the discovery of the first symptoms was so high that no mosaic was evident in the uncovered plants in near-by pots or in the field. Just prior to July 22 there were several foggy days, which resulted in the lowering of the daily maximum temperature by several degrees, and mosaic had reappeared in the plants in the field and in pots on this date.

Usually, several alfalfa seedlings were growing in a pot, and the tops soon became so intermingled that accurate counts were difficult to make, especially since these plants were always covered by cages. Rather than remove the cages and expose the plants to insect attack while the stems were being untangled and the plants counted, it was decided to make as accurate an estimate as possible without removing the cages. For this reason, the numbers of plant given in the table, where more than one or two are involved, are only approximately correct. The figures obtained show that out of a total of 74 plants 27, or 36.5 per cent, became infected.

TABLE 1.—*The results of inoculating alfalfa plants with mosaic, using aphids as vectors on six different dates in 1930*

Experiment number	Date inoculated	First symptoms apparent on	Approximate number of plants inoculated	Approximate number of plants infected
417	March 20	April 2	2	1
421	April 7	July 22	20	5
422	April 10	April 18	20	2
454	Sept. 27	Oct. 4	10	10
455	Oct. 1	Oct. 13	2	2
458	Oct. 11	Oct. 21	20	7

DISCUSSION

In those plants that became infected in these experiments, mosaic symptoms usually appeared first in one or at most only a few of the leaves present

when the inoculations were made, but those leaves that developed subsequently commonly showed infection. Although not so high a percentage of infection was obtained in some experiments as might have been expected or desired, yet the data presented indicate pretty conclusively that alfalfa mosaic can be transmitted by the pea aphid. It is thought that a higher percentage of infection would have been obtained had the experimental conditions been more favorable for the development of the disease. As no greenhouse space was available, the experiments were all conducted outdoors. It was very difficult to find a time when all conditions were suitable. For example, when the plants were in the proper stage of growth for inoculating, the temperature might be too high or too low, or when the temperature was right the plants were not. That temperature is a factor in the appearance of mosaic symptoms, and probably in infection also, is suggested by the fact that the disease is not evident in plants growing in the field during either hot or cold weather, but may be very conspicuous in 100 per cent of the plants during moderately cool periods, such as occur in the spring and autumn months, or in the summer during prolonged foggy periods. Light also may have been a factor in the appearance of the mosaic symptoms. It will be noted in table 1, that infection was obtained in March, April, September, and October. Similar experiments conducted in December, January, February, and May gave negative results.

The plants used in some of these experiments were not in the best condition for infection with mosaic or for its continued appearance, since they did not do well under cages and the leaves often became badly affected with a leaf-spot (*Pseudopeziza medicaginis* (Lib.) Sacc.) or with rust (*Uromyces medicaginis* Pass.).

It is possible that other insects will be found to transmit the disease more readily than the species of aphids used. That the disease is insect-transmissible is indicated also by the fact that plants held under cages for an indefinite period remained free of mosaic, while other plants to which insects had free access usually became diseased when quite young. That the virus is perennial in the tissue was shown by keeping two affected alfalfa plants caged for 2 years. The tops were cut off several times during this period and the new leaves always had more or less mosaic. No evidence that the disease is present in the seed or soil was obtained. Although over 100 different strains and varieties of alfalfa have been under observation, no indication of resistance has been seen in any of them.

F. R. Jones, in a letter dated June 30, 1930, stated that he also apparently had obtained a small percentage of infection by placing aphids from alfalfa plants affected with mosaic onto healthy plants.

A POSSIBLE SECOND VIROSIS IN ALFALFA

As already mentioned, F. R. Jones sent a number of alfalfa plants affected with mosaic from Wisconsin to the writer for comparison with the California strain. All of these plants, with one exception, exhibited mosaic symptoms identical with those of similarly affected plants in California. The leaves of the excepted plant were more crinkled and the spots were very small and lacked the characteristic rings. The symptoms of this disease are shown quite well in the picture of 2 of the twigs from the plant in figure 1, B. The general appearance of the plant suggested the presence of a virosis of the mosaic type but different from the mosaic discussed in this paper. A single unsuccessful attempt was made to transmit the disease by means of aphids. In a letter accompanying the plant, Dr. Jones stated that it had grown near many other kinds of plants affected with mosaic. It seems probable that some other virus had been transmitted to this alfalfa plant. Field observations have suggested the existence of still other mosaic-like viruses capable of attacking alfalfa.

SUMMARY

A transmissible virosis of alfalfa, of the mosaic type, is described and illustrated. The disease has been transmitted by means of the pea aphid. The existence of what appeared to be a slightly different type of mosaic on alfalfa also is noted.

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OCCURRENCE OF TOBACCO RING-SPOT-LIKE VIRUSES IN SWEET CLOVER[1]

R. G. HENDERSON

(Accepted for publication May 24, 1933)

INTRODUCTION

Earlier reports (1, 2, 6) have indicated that there may be some connection between the occurrence of ring spot in the tobacco crop and the growing of sweet clover in tobacco rotations, or to the presence of sweet clover in the vicinity of tobacco fields. Such a relationship is indicated by the fact that ring-spot infection is often very severe in tobacco following a sweet-clover crop. No experimental work, however, has been done to determine whether the sweet clover is directly responsible for this increase in the amount of disease.

REVIEW OF LITERATURE

Wingard (6) reported that sweet clover, *Melilotus officinalis* Lam., was susceptible to the tobacco ring-spot virus, and that it occurred naturally on sweet clover. The natural occurrence of the tobacco ring-spot virus on sweet clover has been reported also by Henderson and Wingard (2). Fenne (1) stated that typical ring spot of tobacco was obtained from sweet clover, *Melilotus alba* Desr., on several occasions. Young[2] reported 30 per cent infection of white sweet-clover plants with ring spot in a one-fourth acre field in Montana.

OCCURRENCE OF RING-SPOT-LIKE VIRUSES ON SWEET CLOVER

It is difficult to estimate the extent of the natural occurrence of viruses on sweet clover that will produce symptoms on tobacco similar to ring spot. Such virus-infected sweet-clover plants cannot always be positively identified by symptoms, alone. Plants have been collected exhibiting symptoms that were presumed to be caused by the ring-spot virus, but, when such material was used as inoculum on tobacco, no infection was obtained. Abnormal sweet-clover plants, however, have been collected in various parts of Virginia that were definitely infected with a type of ring-spot virus. The inoculation of tobacco plants with juice from these abnormal plants has resulted in the production of ring-spot-like symptoms. At first, the viruses obtained from sweet clover were considered identical with the tobacco ring-spot virus, but

[1] Paper No. 81 from the Department of Botany and Plant Pathology, Virginia Agricultural Experiment Station.

[2] Young, P. A. Ring spot of white sweet clover in Montana. U. S. D. A. Bur. Pl. Ind. Pl. Dis. Reptr. **14**: 125. 1930.

a closer examination of the symptoms produced on tobacco by these viruses indicates that they may be different from it. Henderson and Wingard (2) stated that the only significant difference between the sweet-clover viruses and the tobacco ring-spot virus was in the intensity of the symptoms produced on tobacco. The viruses from sweet clover seemed to be attenuated forms of the tobacco ring-spot virus. In some instances inoculations on tobacco with a sweet clover extract produced primary lesions that could have been considered identical with primary lesions of ring spot, but the secondary symptoms produced by systemic infection proved to be very mild. In most cases the sweet-clover virus was lost in subsequent transfers. The illustrations shown in the previous publication (2) were of a disease on tobacco caused by a sweet-clover virus that appeared to be less attenuated than the majority of the other viruses obtained from this host.

Most of the observations on sweet clover have been made when the plants were immature; therefore, it was not always possible to determine whether they were of the yellow or white species. Wingard's work was with the yellow species (*Melilotus officinalis*), but in all probability the majority of the specimens collected on different farms by the writer were of the white species (*M. alba*), since it is the one commonly used commercially in Virginia.

The ring-spot-like virus on sweet clover appears to be in a more virulent form during early spring; in fact, the virus seems to become inactivated in the summer. Sweet clover plants collected on May 2, 1932, were infected with the new virus disease described below. Additional plants collected about 3 weeks later in the same locality and exhibiting similar symptoms did not carry an infectious virus as shown by inoculations on healthy tobacco. In other instances the writer has been unable to obtain infection on tobacco in midsummer from sweet-clover plants that showed the usual symptoms of virus infection.

A NEW VIRUS ON SWEET CLOVER

In early May, 1932, a specimen of sweet clover was collected near Winchester, Virginia, that appeared to be affected with a virosis. The leaves showed chlorotic markings of various shapes (Fig. 1, B and C). The chlorotic areas often followed the midrib of the leaflets and extended along the lateral veins towards the margin. Some leaflets showed chlorotic blotches and spots of various sizes. Very often the markings were of fine lines that followed along the veins or ran in irregular circles. The younger leaves were severely dwarfed and deformed with much puckering and twisting of the bud leaves. This characteristic is noticeable in the two specimens on the left in figure 1, D. The specimen on the right in this picture is from a healthy plant. Figure 1, A, is a leaf from a healthy plant.

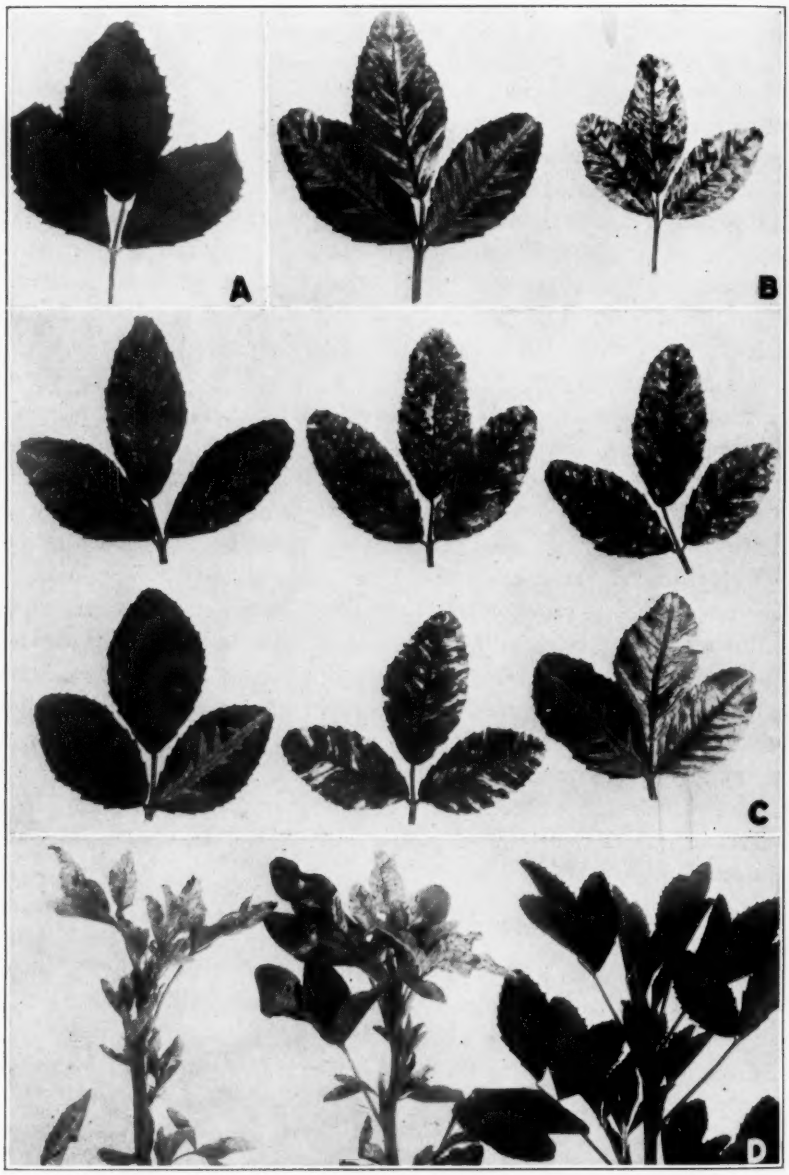

FIG. 1. Sweet clover showing symptoms of the new virus disease. A. Healthy leaf. B and C. Leaves showing chlorotic markings. D. Shoots showing dwarfing and distortion of leaves as compared with healthy specimen on right.

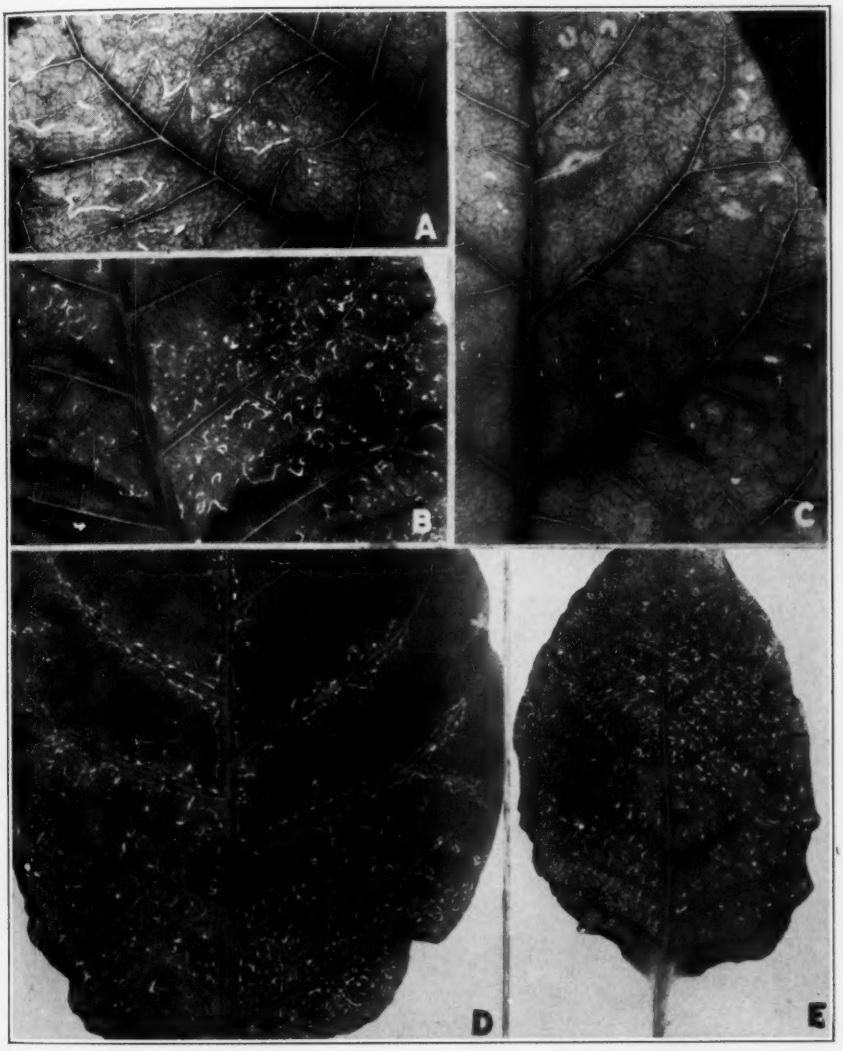

FIG. 2. Symptoms on Turkish tobacco produced by the new virus from sweet clover. A. Portion of leaf showing necrotic zigzag lines (slightly enlarged). B, D, and E. More severe forms of symptoms. C. Faint chlorotic markings on young leaf without the presence of necrotic lesions.

Inoculation of Tobacco

Leaves taken from the abnormal sweet-clover plants were ground in a mortar with a little water and used to inoculate healthy Turkish tobacco plants in the greenhouse. A few faint rings or spots appeared on the in-

oculated leaves in about 6 days. A little later systemic infection developed and it was observed that the symptoms were quite different from those that had been obtained from previous inoculations with sweet-clover extracts. The first noticeable expression of systemic infection was a few faint chlorotic or necrotic zigzag lines following, more or less, some lateral vein (Fig. 2, A). Still fainter contour-like lines could be seen in the tissue when the leaf was held to the light. By very close examination these "contour" marks can be seen in figure 2, A and C.

On certain leaves the symptoms became more pronounced as shown in figure 2, B, D, and E. The nature of these symptoms varied considerably. Chlorotic or necrotic lines followed along the veins on some leaves. On other leaves the lines were in the form of rings, or followed the smaller veins, cutting off the intercostal tissue into islands of irregular shape. On some of the more severely affected leaves the tissue along the veins was a darker green than the rest of the leaf. A part of the leaf in figure 2, E, shows this symptom.

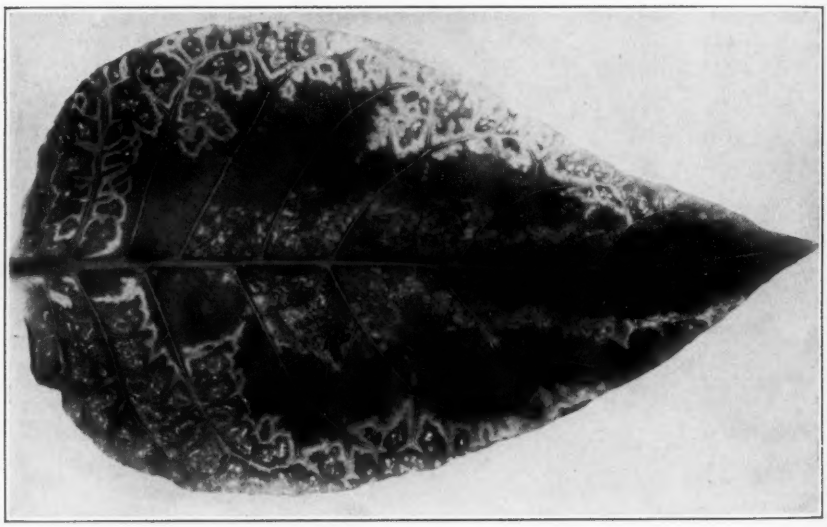

FIG. 3. Leaf of Turkish tobacco showing chlorotic or bleached lines and blotches produced by the new sweet-clover virus.

At times broad chlorotic or bleached lines appeared that were unlike those described above. (Fig. 3.) These bleached lines followed the veins, more or less, and were usually near the leaf margin. Yellow blotches also were present on the central part of the leaf. This bleached type of symptom usually appeared on the upper leaves and was first noticed on leaves that nearly touched the glass roof of the greenhouse in which the plants were growing.

It is of interest to note that the symptoms described above did not occur on all of the leaves. Some of the leaves apparently were healthy, while others, immediately above and below them, showed symptoms. Tobacco plants affected with this virus from sweet clover did not appear to mask the symptoms

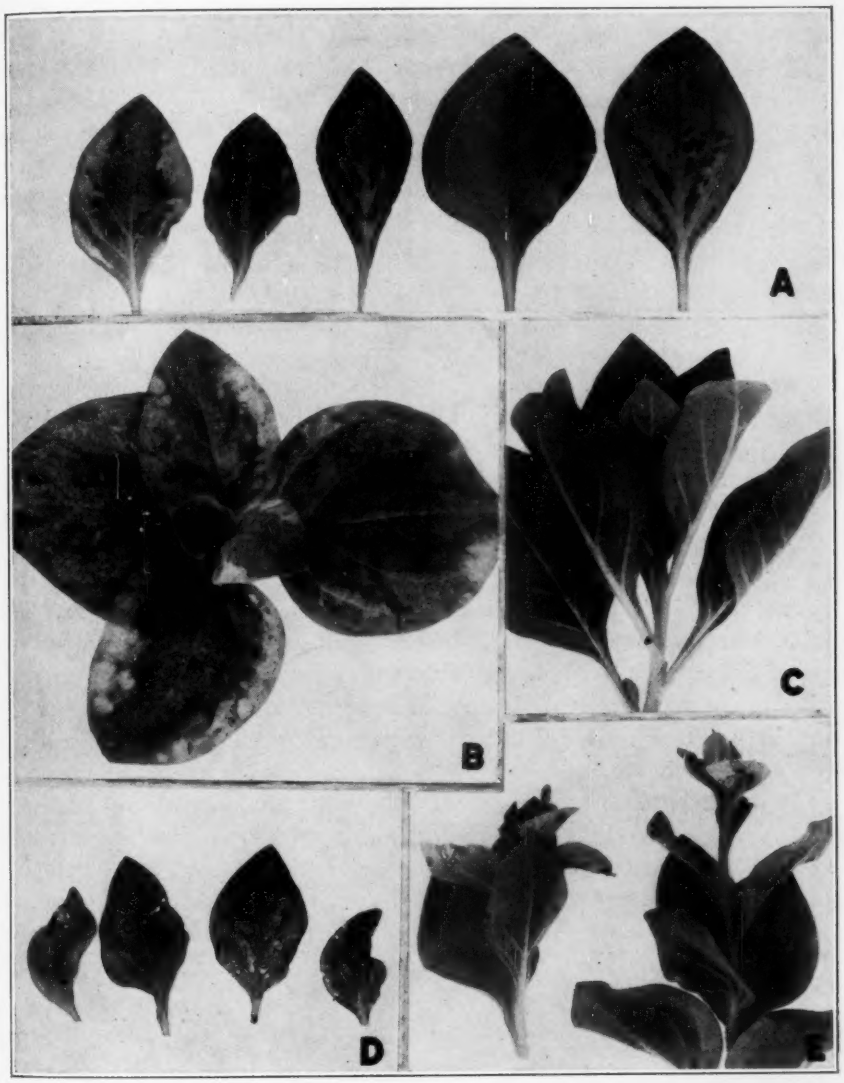

FIG. 4. Symptoms of sweet-clover-virus infection on petunia. A and B. Leaves showing chlorotic streaks and blotches. C. Shoot from healthy plant. D. Leaves from diseased plant showing more severe or necrotic type of symptoms. E. Shoots from diseased plants showing necrotic lesions on bud leaves.

permanently or develop an immunity from it, such as that described for true tobacco ring spot by Wingard (6) and also by Price (4).

Inoculation of Petunia

Healthy petunia plants were inoculated with the clover virus. No signs of infection were noticed on the inoculated leaves, but in about 10 days symptoms began to appear on the new leaves. The leaves were chlorotic in areas along the margin and in spots and blotches in other parts. (Fig. 4, A.) On some leaves pale green areas extended along the midrib and out between the veins. The whole leaf in some cases became yellow to light green with the exception of a few dark green spots or streaks near the base. In other cases the chlorosis was in the form of small specks along the midrib and veins or over the entire leaf, giving it a gray cast. At times the chlorosis gave the foliage a variegated appearance. (Fig. 4, B.)

The symptoms on petunia did not vary so much as they did on tobacco. None of the leaves appeared to be free of the disease, as was the case with tobacco. Every leaf showed some chlorosis. At times the disease became quite severe on the tips of certain branches. The leaves were dwarfed and deformed. Spots and streaks in the tissue died (Fig. 4, D and E). Figure 4, C, shows a healthy branch of petunia for comparison.

Inoculation of Sweet Clover

White sweet-clover plants growing in the greenhouse were inoculated with expressed juice from tobacco infected with the sweet-clover virus. The results, however, were negative. The inoculations were repeated, but no signs of infection appeared. Thinking that possibly the symptoms were masked or had been overlooked, inoculations were made from the sweet-clover plants back to tobacco, but the results were negative, showing that the plants had not become infected.

The writer has also been unable to obtain infection of white sweet clover with the true tobacco ring-spot virus. As stated above, Wingard obtained ring-spot infection on yellow sweet clover. It would seem from this that there was a difference in the susceptibility of the yellow and white species. The writer believes, however, that his failure to secure infection of sweet clover was probably due to some environmental factor. In work to be reported later, it has been determined that ring-spot infection on tobacco is very greatly influenced by the air temperature in which the plants are growing. It is probable that temperature also is a factor in sweet-clover infection. P. A. Young,[3] who reported natural occurrence of ring-spot on sweet clover in Montana, further reported that he was able to transfer the virus from sweet clover to bean but attempts to inoculate healthy sweet-clover plants were unsuccessful.

[3] In personal correspondence to the writer dated April 5, 1933.

DISCUSSION

The question of the ring-spot disease of tobacco has become more complicated with the discovery of viruses on other host plants that are able to infect tobacco and produce a disease with symptoms very similar, yet not identical, to ring spot. In only a few cases has the tobacco ring-spot virus been positively identified as occurring in nature on other hosts. With the wide distribution of ring spot in tobacco and the occurrence of such a high percentage of infected plants in certain fields, there seems to be some indication that other hosts carry the virus over winter. Evidence so far points to sweet clover as an over-wintering host plant. Nevertheless, it has not been proved that the ring-spot-like viruses occurring in sweet clover are identical with the tobacco ring-spot virus. The relation of the viruses from sweet clover to the tobacco ring-spot virus, therefore, remains to be determined. Johnson (3) and Valleau (5) have described a number of viroses of tobacco that somewhat resemble ring spot. It is clear, however, that the symptoms of the various diseases are not the same and also that the properties of the viruses differ in many respects. These diseases have been considered by Johnson and Valleau as being caused by separate and distinct viruses. On the other hand, the symptoms that the writer has obtained on tobacco by inoculations with viruses from abnormal sweet clover plants have all been similar, though not always identical, and in one case they have been identical (as to virulence) to the symptoms of true tobacco ring spot. It would seem more plausible to classify these sweet-clover viruses as strains (with possibly some exceptions) of one virus rather than consider them as separate viruses.

Failure to obtain infection by artificial inoculation of sweet clover with the ring-spot and ring-spot-like viruses has handicapped this phase of the work. A virus can be transferred from diseased sweet clover to healthy tobacco without difficulty, but the writer has not succeeded in transferring the virus from diseased tobacco back to healthy sweet clover. The environmental conditions necessary for the virus to infect sweet clover seem limited to a narrow range.

It is well known that environment influences the susceptibility of certain plants to diseases. For example, it is extremely difficult to get ring-spot infection on *Nicotiana glutinosa* L., but under certain conditions, which have not been determined, infection of this plant with ring spot can be secured. It is possible that if the proper environmental conditions prevail, infection can also be obtained on sweet clover with the ring-spot virus.

SUMMARY

The natural occurrence of ring-spot-like viruses on sweet clover is discussed. Viruses of the ring-spot type have been found to occur on sweet

clover in various parts of Virginia. It is suggested that the viruses from sweet clover may be forms or strains of the tobacco ring-spot virus.

A new virosis of sweet clover is reported and the supposed symptoms described. This virus is infectious on tobacco, producing symptoms of the necrotic-pattern type but distinctly different from the symptoms of ring spot. It is also infectious on petunia, producing a type of chlorosis. The virus was not successfully transferred back to healthy sweet clover plants.

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YELLOW RUST OF RUBUS[1]

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(Accepted for publication April 24, 1933)

A general description of the yellow rust of Rubus has been presented in a previous paper (6) in which the causal organism was considered to be *Phragmidium imitans* Arthur. In our consideration of the taxonomy of the species since then, the question of its identity with the European *P. rubi-idaei* (DC.) Karsten has repeatedly arisen. Grove (4, pp. 298–299) briefly mentions *P. imitans* as very similar to the European species, having similarly formed aecia and pycnia.

In order to obtain more direct evidence on this relationship, materials from America and Europe were carefully compared. Thanks to the courtesy of Dr. E. J. Butler, dried specimens of uredia and telia of *P. rubi-idaei* were secured through the British Imperial Mycological Institute. These had been collected in August, 1931, from the wild raspberry, (*Rubus idaeus*) in England. Briosi and Cavara Exsiccate No. 130,[2] in the Herbarium of Oregon State College, supplied aecial material from Italy, to compare with Oregon specimens. We have been able to detect no differences between this European and Oregon material and have concluded that the Oregon rust is identical with the European and should bear the older name, *Phragmidium rubi-idaei* (DC.) Karst. The same conclusion was reached by Dr. J. C. Arthur, according to a recent communication.

The present study (1) has aimed to follow the complete life history and morphology of the rust by means of infection from artificial inoculation in the greenhouse and natural infections under field conditions, (2) to determine from greenhouse and field observations the relation of certain ecological factors to infection and epidemics of the disease, and (3) to compare the susceptibility of certain species and varieties of Rubus to the disease.

LIFE HISTORY AND MORPHOLOGY

Since the Cuthbert red raspberry is known to be very susceptible to yellow rust, this host plant was used for inoculation studies in the greenhouse and for observations concerning the life history of *Phragmidium rubi-idaei* in the field. Inoculations in the greenhouse were made in a tent-like humidifier where a moisture-saturated atmosphere could be maintained.

[1] Published as Technical Paper No. 197 with the approval of the Director of the Oregon Agricultural Experiment Station. Contribution from the Department of Botany and Plant Pathology.

[2] Briosi, G., and F. Cavara. I Funghi Parassiti, Exsicc. Fasc. VI: No. 130. Pavia. 1891.

Potted plants to be inoculated were previously placed in the humid chamber for 12 hours so that the leaves were in the best possible condition for infection. Then spores of the desired stage were dusted or brushed on certain marked leaves and the plants allowed to remain under the moist conditions for 72 hours after inoculation. Then they were returned to ordinary greenhouse conditions. The work began with teliospore germination and sporidial infections in the greenhouse.

Teliospores and Their Germination. Teliospores first appear under field conditions early in July and increase in quantity during the summer, even through very dry weather. By late September or early October the telial stage is the only one to be found, the lower surface of infected leaves having a sooty black cast due to the abundance of teliospores. If the raspberry leaves fall undisturbed they alight bottomside up, exposing a maximum number of the telia—a very important natural behavior in the economy of this stage of the rust.

The teliospores of *Phragmidium rubi-idaei* require a considerable rest period before they will germinate in quantity. Several attempts to germinate them in hanging drops failed early in the autumn, even after exposure to freezing temperatures, and the first successful germinations were secured on living leaves in the greenhouse during January, although previous attempts had failed. Simultaneously, difficulty was experienced in obtaining germination in water. Evidently, the host exerts a stimulating influence on teliospore germination, and the conditions affecting our water-germination experiments did not simulate closely enough the natural conditions prevalent from March to May, when sporidial infection normally takes place.

From greenhouse inoculations with teliospores it was discovered that pycnia make their first appearance in 10 to 13 days after the spores were placed on the leaves. Under field conditions the first leaves of the Cuthbert red raspberry unfolded at the base of some marked fruiting canes on March 15. On March 25 and 26 pycnia were first recognized on the lower leaves of these canes, in close proximity to teliospore masses on old leaves that had over-wintered on the ground, and were especially abundant where dead leaves had accumulated. The first infection period was accompanied by a period of prolonged wet weather and moderate temperatures, after which pycnia and aecia began to appear.

A histological and cytological study of the rust through its complete life history was carried out by the junior author[3] who found the same story

[3] This histological and cytological study, along with the field and host susceptibility studies presented in this paper, comprised a subject assigned to the junior author for a thesis in partial fulfillment for the degree of Master of Science, June, 1932. This complete thesis, with full data, is filed in the Library of Oregon State Agricultural College, Corvallis.

for *P. rubi-idaei* as that presented by Blackman (2) for *P. violaceum* and by Christman (3) for *P. speciosum,* except that in these species the pycnia are on the opposite side of the leaf from the aecia, while in *P. rubi-idaei* the aecia immediately surround the pycnia in a sorus that completely encircles them (4, 6). Whether the aecia and pycnia arise from the same mycelium is a question, but in the early stages of aecial development the primordial mycelium and buffer and stalk cells are uninucleate. It appears that the first binucleate cells occur in the erect aecial hyphae.

RELATION OF MOISTURE AND TEMPERATURE TO WAVES OF INFECTION

The climatic factors influencing spore germination and leading to epidemics of the various stages of yellow rust are worthy of brief consideration. Among other climatic factors, moisture and temperature are evidently most responsible for waves of aecial and uredial production, moisture being the prime factor. Our records show that any climatic condition, such as rainfall and fog or other forms of very high humidity, which result in the formation of films of free water on leaf surfaces, is conducive to infection by yellow rust, provided these moisture conditions continue for several hours at a time.

In order to study the progress and numerical intensity of the occurrence of aecia and uredia during the spring and summer of 1931, 24 noninfected leaves were labeled. At each interval of 7 days these leaves were carefully observed for the first appearance and progress of rust infections. By this method it was found that infection took place during moist weather. With each repetition of such weather, the number of infections increased to a maximum and then, with drier weather, infection abruptly ceased. At each of the intervals at which infection ceased, new sets of noninfected leaves were labeled and observed for infection. During the period of investigation in 1931, the weather conditions resulted, therefore, in an intermittent series of 2 aecial and 2 uredial waves of infection. (Fig. 1.)

Figure 1 is a graphic presentation of the relative humidity, rainfall in inches, and temperature, correlated with the progress of waves of aecial and uredial production in terms of the average number of new infections. The climatological data used in this study are those taken by the U. S. Weather Bureau at 5 p. m. daily at Corvallis, Oregon. After the first appearance of pycnia on March 25 there was a steady increase in the number of aecia, which reached a maximum on May 7. This increase of aecia followed immediately after the more or less constant rainfall from March 6 to April 14. It constituted the major infection wave that year and definitely established rust in the fields. The next wave of aecial infection started about May 15 and reached a maximum about June 3. This was not so severe as the first wave, undoubtedly because of the generally lower level

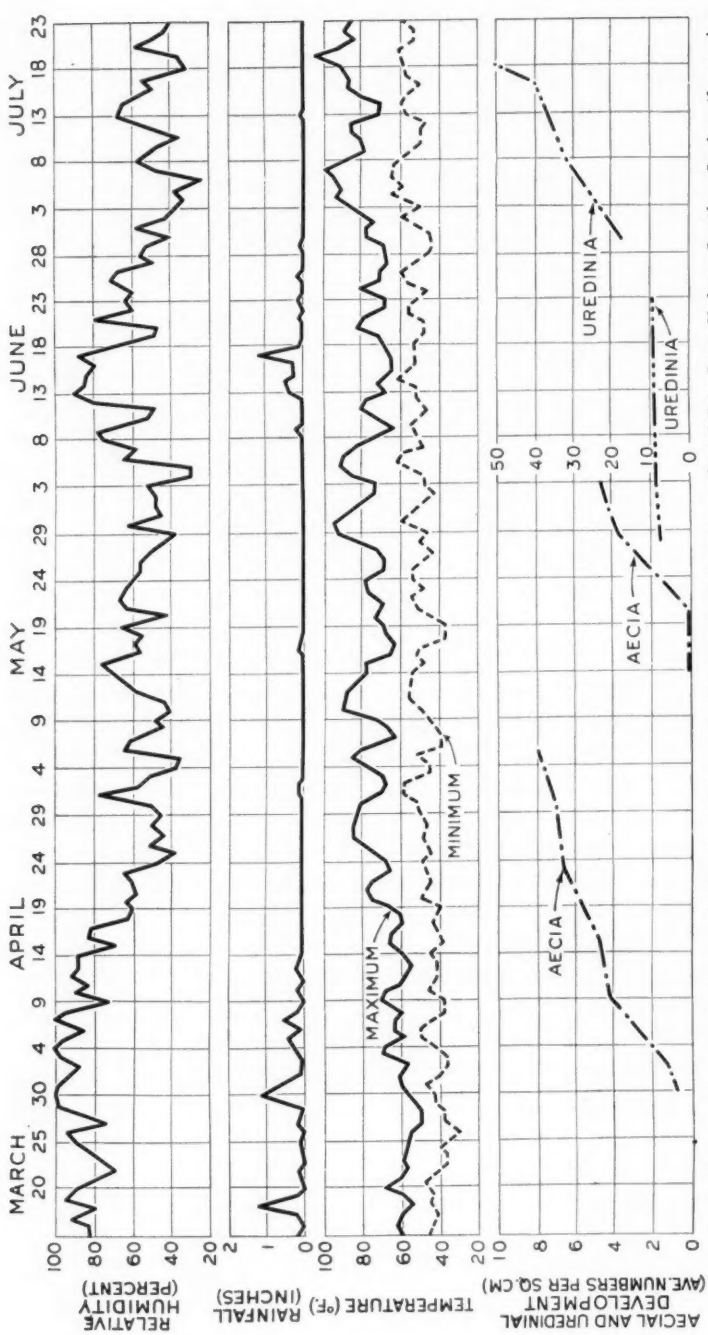

FIG. 1. Relation of precipitation, relative humidity, and temperature to waves of aecial and uredial production during the spring and summer of 1931, Corvallis, Oregon. The development of aecia and uredia is expressed in terms of the average number of new infections per square centimeter of leaf surface.

of relative humidity and less rainfall, accompanied by somewhat higher temperature during this second period. The slight drop of temperatures from May 15 to 27 accompanied by rainy weather during May 16 to 18, inclusive, was conducive to aecial production, which followed into June, when uredial sori first became noticeable.

The uredia very gradually increased from late May to mid-June but the one major wave of infection resulted from the slightly lower temperatures and higher humidities accompanying the rains from June 8 to 29. This wave of uredia started in earnest about June 28 and reached a maximum about July 21 (Fig. 1). Relatively high humidity, cool temperature, and slight precipitation from July 12 to 15 and, again, from September 5 to 10, produced rather continuous but slight waves of uredia following both periods.

Figure 1 shows graphically the more conspicuous waves of aecial and uredial infection during the spring and early summer of 1931. In the summer of 1932 there was rainfall each month. Each rainy period was followed by rapid increase of the prevailing stages of the rust and resulted in a cumulative severity of infection throughout western Oregon, greatly surpassing that of 1931.

Sporidial infections on Cuthbert raspberry in the greenhouse were brought about in 2 ways, by maintaining a moisture-saturated atmosphere in a closed chamber or by spraying moisture on the leaves in a humid chamber. Free water on the leaf surface is more readily maintained with a slight drop in temperature and high humidity. Under these conditions, mature aecia appeared in 10 to 14 days after inoculation, initial stages appearing in 6 to 7 days.

Inoculations made on the upper surfaces of certain leaves and on the lower surfaces of others reveal the fact that sporidia apparently infect one surface as readily as the other, irrespective of the number of stomata. Stomata are rarely found on the upper surface of Cuthbert raspberry leaves, but aecia are usually found more abundantly on the upper side under field conditions. Undoubtedly, under natural conditions the sporidia are for the most part borne by air currents in such manner as to alight on the upper surface of leaves, since greenhouse experience has shown that the aecia appear on the surface inoculated with sporidia.

Further, greenhouse inoculations with aeciospores and urediospores have shown mature uredia resulting in 8 to 13 days after inoculation, provided the same environmental conditions necessary for infection by sporidia were maintained. There is, however, one essential difference. Uredia always appear first directly below stomata on the lower surface of the leaf. All inoculations by aeciospores and urediospores on the upper surface of leaves were unsuccessful. These infections always take place through stomates.

Thus, by correlating the greenhouse experiments with the field observations shown in figure 1, it is readily seen that under natural conditions the lag of the waves of infection after a rainy period is doubtless due to the incubation period from incidence of natural inoculation until infections become apparent; also, that rainfall, in itself, supplies free moisture on leaf surfaces or is usually accompanied by slight drop in temperatures and sufficiently high humidities to bring about the necessary free moisture from fog or dew. These are pertinent facts in the prediction of epiphytotics of the rust caused by *Phragmidium rubi-idaei.*

SUSCEPTIBILITY

The Cuthbert raspberry is the one standard commercial red variety grown in the Pacific Northwest. The crop from this variety has several commendable qualities, but the plants, besides their susceptibility to *P. rubi-idaei,* lack other desirable characteristics. Consequently, other varieties are being tested for this locality and new hybrids are now being produced in an effort to find a suitable stock to replace or supplement the Cuthbert. For this reason it was desirable to procure information concerning the susceptibility of available varieties and species of Rubus that might be introduced or used as parents in the production of new hybrids.

Previous references to the disease have reported it in America on *Rubus leucodermis* Doug. (Western wild black raspberry), varieties of *R. occidentalis* L. and *R. strigosus* Michx., and the Salmon berry (*R. spectabilis* Pursh) (1, 6).

In Europe (4) *Rubus idaeus* L. and some of its horticultural varieties are more or less infected. In Saccardo (5) it is reported on *R. strigosus, R. idaeus* and *R. odoratus* from many European countries.

In the fall and winter of 1931–32, the following several species and varieties of Rubus were inoculated to determine their relative susceptibility to *Phragmidium rubi-idaei: Rubus laciniatus* Willd. (Evergreen blackberry), *R. leucodermis* Doug. (Western wild black raspberry), *R. loganobaccus* Bailey (Loganberry), *R. macropetalus* Doug. (Northwest trailing blackberry or dewberry), *R. parviflorus* Nutt. (Thimble berry), and *R. spectabilis* Pursh (Salmon berry). Of the red varieties used, the Antwerp and Lloyd George are supposed to be true horticultural varieties of *R. idaeus* L. and Ranere (St. Regis) a true variety of *R. strigosus* Michx. The probable hybrids of these two species of red raspberries tested include Cayuga, Chief, Cuthbert, Golden Queen, Herbert, Latham, Marlboro, Owasco, and Seneca. A purple variety, Webster, and 3 horticultural varieties of *R. occidentalis,* Cumberland, Munger, and Plum Farmer, were used. Three local species of wild rose, *Rosa gymnocarpa* Nutt., *R. nutkana* Presl., and *R. rubiginosa* L. also were tested to learn whether the yellow rust crosses over to this genus.

Potted plants of all these varieties and species were grown and inoculated in the greenhouse. Ten to 12 varieties were inoculated at a time under identical conditions. In each test the Cuthbert was included as a standard of comparison between the various groups tested. The inoculations were made in humidity chambers, where conditions favorable to infection were constantly maintained. Urediospores, continuously cultured in the greenhouse on Cuthbert raspberry, were used in all the inoculations. After inoculation the plants were incubated for 72 hours in the humid chambers. After removal 6 examinations were made and data taken at the end of 13, 15, 19, 23, 28 and 32 days from the time of inoculation.

After all varieties had been tested once they were divided into susceptible and resistant groups based on the first test. Each group was then tested again in duplicate. In every case the results of the second duplicate tests conformed with and bore out the findings of the preliminary experiments.

The number of infections per unit area of leaf surface was determined by actual count and the area estimated by the well-known method of matching leaflets with leaf-shape cardboards of known area. It was found necessary to take another factor into consideration when estimating the damage to the host. The uredial pustules were much larger on some varieties of Rubus than on others. Since the size of the pustule seems to indicate to some extent the degree of vigor of the rust on a particular host, this size factor was included in the estimate of degree of susceptibility of one plant compared with another.

The results of this inquiry into the susceptibility of host plants to infection by *Phragmidium rubi-idaei* can be summarized briefly. The species and varieties found immune from yellow rust included all of the blackberries tested, *Rubus parviflorus, R. spectabilis,* the Munger and Plum Farmer varieties of *R. occidentalis,* and the three roses *Rosa gymnocarpa, R. nutkana,* and *R. rubiginosa.*

All infection counts and general characters brought about by infection of the varieties showing degrees of susceptibility are given in table 1. The numbers indicate infections per square centimeter of leaf surface and the letters refer to the comparative sizes of the pustules, L indicating large, M medium, S small, and VS very small. On the 28th day after inoculation telia were observed. The column designated telia refers to their degree of abundance, H indicating heavy, M medium, S slight, and N none. The varieties are placed in the order of their susceptibility as arbitrarily determined by the intensity of infection and the size of the uredia.

The significant results presented in this table, so far as desirable red raspberries are concerned, are the extreme susceptibility of the Cuthbert and Marlboro, and mere disease tolerance of the Lloyd George. It is of

TABLE 1.—*Study of relative susceptibility of certain varieties and species of Rubus as indicated by greenhouse tests*

Host	Period after inoculation					
	13 days	15 days	19 days	23 days	28 days	32 days
	Number of infections per sq. cm. of leaf surface				Telia	Condition of plant
Marlboro	12.4 L	19.4 L	65.6 L	80.8 L	S	Heavy infection; 100% of spots necrotic.
Cuthbert	7.6 S	16.8 L	39.9 L	67.8 L	H	Heavy infection; 100% of spots necrotic.
Rubus leucodermis	2.8 S	12.7 M	31.9 L	39.6 L	M	Medium infection; 50% of spots necrotic, others yellowed.
Golden Queen	2.9 M	9.9 L	26.8 L	35.8 L	M	Medium infection; 20% of spots necrotic, others yellowed.
Webster	1.5 S	5.4 S	33.5 M	43.5 M	S	Medium infection; 5% of spots necrotic, others yellowed.
Herbert	1.1 S	2.8 M	11.7 M	30.9 M	N	Slightly affected; spots yellowed.
Cayuga	0.1 S	2.5 S	17.7 S	29.6 S	S	Slightly affected; spots yellowed.
Ranere	4.2 S	6.5 S	14.8 S	21.7 S	M	Slightly affected; spots yellowed.
Antwerp	3.6 S	6.1 S	5.4 S	18.4 S	H	Slightly affected; spots slightly yellowed.
Seneca	0.0	0.0	2.5 S	17.7 M	S	Slightly affected; spots slightly yellowed.
Owasco	0.0	0.0	6.0 S	10.0 S	N	Slightly affected; scarcely spotted.
Cumberland	0.0	0.1 VS	2.8 VS	6.3 VS	N	Very slightly affected; scarcely spotted.
Lloyd George	0.0	0.0	3.6 VS	5.4 VS	N	Very slightly affected; scarcely spotted.

interest to observe that the Lloyd George and Antwerp, varieties of *Rubus idaeus* are much less tolerant of the disease than most of the hybrid crosses of *R. idaeus* with *R. strigosus*. The two hybrids, Chief and Latham, are quite highly resistant. According to these tests, the Cuthbert is far more susceptible than the Ranere, but, under very favorable climatic conditions near the coast in California, the latter, the standard commercial variety in central California, is much more severely damaged than the Cuthbert under the most favorable conditions for the disease in western Oregon and Washington. Thus, the data in table 1 are presented with the reservation that

the comparative susceptibility of host plants, as indicated from greenhouse tests, may not in all cases be borne out by actual field experience.

SUMMARY

This paper presents a continuation of our studies with the yellow rust of raspberry, which we are now convinced is caused by the European fungus, *Phragmidium rubi-idaei* (DC.) Karsten. Life history and morphological studies show it to conform closely in essential stages with those of other species of Phragmidium previously described by Blackman and Christman.

Precipitation and other factors, such as high humidity, simultaneously with slight lowering of temperatures, which bring about free-water on the leaf surface, are conducive to infection by the sporidia, aeciospores and urediospores.

The relative susceptibility of about 30 varieties and species of Rubus was tested in the greenhouse. Suffice it to mention here that, among the red raspberries, the Chief and Latham are resistant, the Lloyd George is disease-tolerant, the Antwerp, Owasco, and Seneca are fairly resistant, while the Marlboro, Cuthbert, Golden Queen, Herbert, Cayuga, and Ranere are susceptible in the order named.

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FUNGI AND BACTERIA AS INDICATORS OF THE EFFECTS OF PETROLEUM OILS ON APPLE LEAVES[1]

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INTRODUCTION

An investigation of the effects of petroleum oils and oil sprays on apple leaves made it desirable to find a convenient laboratory method for predicting toxic effects of such agents on the leaves. Hence, experiments were conducted to find an organism that would indicate such toxic effects while it was growing under oils. Fourteen species of fungi and bacteria were inoculated separately onto agar and covered with 22 petroleum oils in nearly 800 cultures and the behavior of each organism was observed and recorded.

REVIEW OF LITERATURE

Söhngen (8) reported that species of mycobacteria oxidized benzene, petroleum, paraffin oil, and paraffin to fatty acids, CO_2, and water, using the organic compounds as sources of carbon and energy. Young (12) reported that 5 species of fungi grew in undiluted oils. Young and Morris (13) used oil sprays made with many of the oils described in figure 2. Lipman and Greenberg (5) found a coccus in petroleum that decomposed petroleum. Tomkins (10) observed that fungi penetrated vaseline. Hopkins and Chibnall (3) grew *Aspergillus versicolor* on 5 synthetic paraffins including $C_{23}H_{48}$ and $C_{34}H_{70}$, and on paraffin wax in mineral solutions. Stieglitz (9) mentioned that cutting oils harbored *Staphylococcus aureus,* and that paraffin oil was a favorable culture medium for such suppurative organisms.

Solubility of oxygen in paraffin oil was given a Bunsen-absorption coefficient of 0.114 at 18° C. by Washburn (11). This is calculated as meaning a concentration of 0.0185 per cent oxygen by weight in the oil; Transformer Oil and Russian Petroleum had nearly 0.025 and 0.03 per cent oxygen, respectively.

Parish (6) and Birkhaug (1) preserved delicate cultures of bacteria for 8 to 24 weeks in media under layers of liquid paraffin. Smith (7) reported that toxic oils inhibited the growth of *Fusarium cubense.* Jordan (4) stated

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Thanks are due to Professors H. E. Morris and F. B. Cotner for their valuable suggestions, and to the Standard Oil Co. (Cal.), Shell Oil Co., and L. Sonneborn Sons, Inc. (N. Y.) for furnishing the oils and records of the viscosities and sulphonatable residues of the oils.

that most chromogenic bacteria require oxygen for pigment production, and lack their pigments in anaerobic cultures.

MATERIALS AND METHODS

The oils used in this investigation are described in figure 2. They are 0 to 44 per cent sulphonatable. Nearly all of them were autoclaved in closed containers to prevent steam from entering and condensing in the oils. They were protected from contaminations.

Corn-meal agar and potato agar were used as slants, and as blocks with dimensions of $10 \times 12 \times 2$ mm. in test tubes. Usually within 0.5 hour after inoculation and before evident growth occurred, undiluted oils were poured separately into the test tubes until the media were covered to depths of 1 to 5 cm., leaving layers of air 1 to 5 cm. thick between the oils and the cotton or cork stoppers. For comparison, some organisms were allowed to grow perceptibly before oils were poured on them. As checks for each series, organisms representing all inoculations were grown on the agar without oil. Cultures were grown at 20° to 25° C. in the laboratory. Notes were taken usually 3 and 5 days after inoculation.

EXPERIMENTAL RESULTS

Fungi Growing in Oils

Rhizopus nigricans Ehr. The minus strain was used in most of the tests. The plus strain was employed in some of the tests, and it reacted like the minus strain. *Rhizopus* produced many sporangia in the oils that permitted luxuriant growth (Fig. 1, A, B.). It produced sporangia and long hyphae in Oil 24 saturated with Oil Red 0. Some of the hyphae in oils had irregular sheaths (fig. 1, B). *Rhizopus* grew poorly in n-decane, which was tested as a representative of the hydrocarbons in petroleum oils. It produced numerous long hyphae in oils. Its tolerance to oils resembled that of apple leaves except that Rhizopus was more tolerant to Oils 1, 2, 5, and 27, which are 20 to 44 per cent sulphonatable. It was the most useful fungus tested for predicting the toxic effects of oils on apple leaves.

Mucor glomerula (Bain.) Lend.[2] was isolated, in 1929, from abundant hyphae that appeared on staminal hairs of *Zebrina pendula* Schn., which were left on a microscope slide in Oil 3 under a cover glass for 8 days. These hyphae were guttulate, 1 to 5 μ in diameter, and most of them had sheaths 10 to 15 μ thick. Some branch hyphae without sheaths protruded from the sheaths. This Mucor grew well in many oils (Fig. 2) and sporulated in the least sulphonatable oils. It produced mycelium 10 to 20 mm. long in Oil 24 and grew well in Oil 24 saturated with Oil Red O stain, and in a mix-

[2] Classified by Dr. A. H. Povah, Farlow Herbarium, Cambridge, Mass.

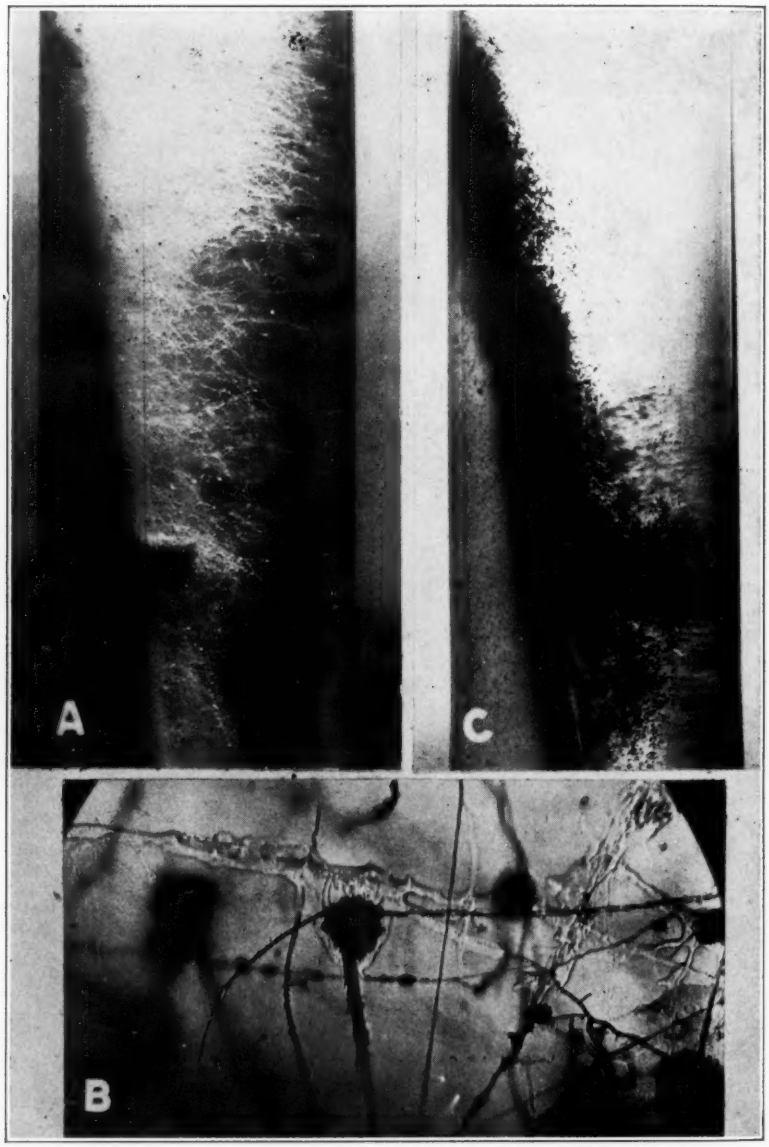

FIG 1. A and B. Rhizopus nigricans (minus strain) grown for 39 days on an agar slant submerged in Oil 3. Hyphae and sporangia projected into oil 13 mm. from the agar. Note sheaths in B. A, ×3; B, ×50. C. Helminthosporium grown for 39 days on an agar slant submerged in Oil 24. ×3.

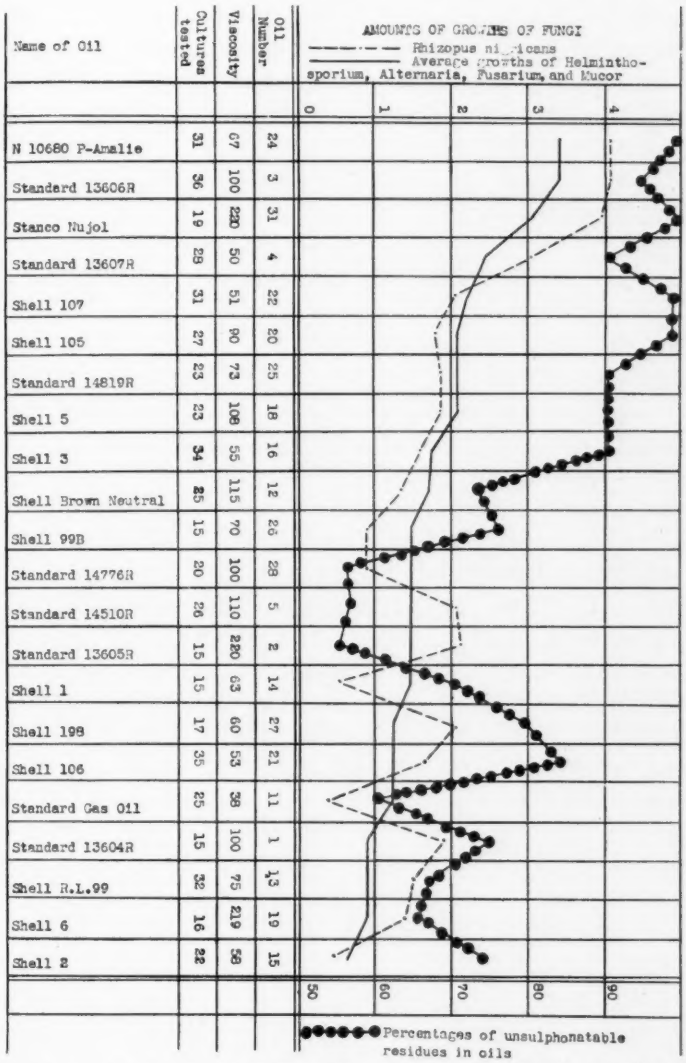

FIG. 2. Amounts of growth of 5 species of fungi in oils compared with the unsulphonatable residues of the oils. The points determining graph lines are placed at horizontal centers of the rectangles. ORDINATE (*left side*): Amounts of growth of these fungi in oils are indicated by numbers that signify as follows: 0 = no growth; 1 = traces of growth; 2 = moderate growth; 3 = abundant growth; and 4 = luxuriant growth with sporulation. A little emphasis was given to rapid growth within 3 days after inoculation. The standard for each fungus was its maximum growth in oils. ORDINATE (*right side*): The percentage of unsulphonatable residue in each oil. These percentages were determined by the oil companies, except for Oils 5, 13, 15, 16, and 21, which were determined by Green (2). ABSCISSA: The *lower line* gives names of oils, and symbols indicating names of oil companies; these signify as follows: *Standard* = Standard Oil Co. of California; *Shell* = Shell Oil Co.; *N* = L. Sonneborn Sons, Inc., New York; and *Stanco* = Stanco, Inc.

ture of ¾ Oil 24 and ¼ n-decane. *Mucor glomerula* grew best in Oils 3, 24, 4, 31, and 5, in the order listed. It did not grow in Oils 16 and 27, probably because they contained intolerable chemicals. It was nearly as useful as *Rhizopus nigricans* in predicting effects of oils on apple leaves.

Achlya conspicua Coker produced long hyphae in nearly unsulphonatable oils, but it grew too sparsely to indicate clear differences in oils more than 11 per cent sulphonatable.

Helminthosporium sativum P., K. and B., grew well in many oils and produced abundant spores in nearly unsulphonatable oils (Figs. 1, C; 2). Hyphae lived in Oil 24 for 227 days and were nearly normal in color, size, and shape, but were very guttulate. Some hyphae bore sheaths in Oil 24. Helminthosporium mycelium in Oil 3 was 15 mm. long. It grew best in Oils 3, 24, 21, 5, 11, 4, 31, and 15, in the order listed, and grew very little in Oils 16, 18, 26, and 27. It grew well in n-decane, but differed from apple leaves in its tolerance for n-decane and Oils 5, 11, 15, and 21.

Alternaria tenuis Nees produced similar mycelium in air and oils. It grew well in n-decane and all of the oils except Oils 2, 13, 15, and 19 (Fig. 2). It produced hyphae 1 cm. long near the glass tube in a culture under Oil 25. It grew best in Oils 24, 31, 3, 4, 18, 26, and 28, in the order listed, but differed from apple leaves in its tolerance for n-decane and Oils 26 and 28.

Fusicladium alopecuri E. and E. grew too slowly and produced hyphae only 1 to 2 mm. long, which were too short to be valuable in indicating the toxic effects of oils. It grew best in Oils 24, 3, 31, 4, 22, 21, and 20, in the order listed, but did not grow in Oils 2, 14, and 19.

Cladosporium sp. grew well in Oil 3, but did not grow in Oil 5. It produced hyphae too short to be useful in further tests.

Fusarium sp. Culture 113 was isolated from a wilting carnation, and produced pink mycelium on corn-meal agar and hyaline mycelium in oils. It grew best in Oils 31, 3, 24, 4, and 26, in the order listed (Fig. 2), and produced hyphae 15 mm. long in Oil 3. It was a useful indicator of the toxic effects of oils on apple leaves.

Fusarium sp. Culture 35 was isolated from wheat, and produced white mycelium on corn-meal agar and in oils. It grew best in Oils 31, 24, 3, 12, 18, and 4, in the order listed, but did not grow in Oil 13. It grew in the other oils considered in figure 2, and produced mycelium 9 mm. long in Oil 24.

Aspergillus sp. grew well in many kinds of oil. It produced mycelium 10 mm. long in Oils 24 and 13, and produced some red mycelium in Oil 13.

GROWING BACTERIA UNDER OILS

Chromobacterium violaceum (Bergon.) Bergey *et al.*, showed enlargement of its inocula on agar under Oils 1, 2, 3, 4, 11, 12, 13, 16, 18, 20, 21, 22,

24, 25, and n-decane, but not under Oils 5, 6 [3], and 7 [3]. The colonies did not produce their purple color noticeably under oils, but the inoculum retained much of its purple color in some cultures under all of the oils during 4 to 27 days. However, much of the purple color disappeared from the inocula under oils in some other cultures.

Serratia marcescens Bizio showed prominent enlargement of its inocula on agar under Oils 1, 2, 3, 4, 16, 21, 22, 24, 25, 31, and n-decane, but not on agar under the other oils listed in figure 2. The colonies did not produce their pink color noticeably under the oils, and the pink color disappeared from the inocula.

Sarcina aurantiaca Fluegge showed enlargement of its inocula on agar under Oils 1, 3, 5, 11, and 20, but not under Oils 4, 13, 16, and n-decane. It did not produce its yellow color under the oils, and the yellow color disappeared from the inocula under Oils 4 and 16.

Bacillus subtilis (Ehr.) Cohn showed enlargement of its inocula under Oils 3, 4, 16, 20, 21, 22, 24, and n-decane, but not under Oils 5, 11, 12 and 15.

Achromobacter sp. was isolated from agar submerged in Oil 24. The colonies were white to hyaline, long, radiating, rapidly growing strands. The bacteria were gram-negative rods 3 microns long. This species was tested under the oils concerned in figure 2, and grew on agar under all of them except Oils 1, 2, 5, and 19. It grew most under Oils 16 and 22.

The inocula of these 5 species of bacteria grew well on agar under oils less than 15 per cent sulphonatable, but grew poorly or not at all on agar under oils more than 25 per cent sulphonatable. Their colonies grew too slowly and indistinctly to be valuable in indicating the toxic effects of oils on apple leaves.

Probably too little oxygen was available in the oils for pigment production by *Chromobacterium violaceum*, *Serratia marcescens* and *Sarcina aurantiaca*. They produced their typical colors on the agar without oil.

Oxygen Relationships of Organism Under Oils

The organisms studied under oils were aerobes, so their growths in oils is evidence that the oils contained sufficient oxygen available to them.

Mycelium in oils usually was most abundant and long within 2 to 30 mm. below the upper surfaces of the oils, and often occurred as horizontal mats of hyphae within 2 to 5 mm. below the upper surfaces of oils in test-tubes, probably because some oxygen diffused from the air into the oils. Hyphae of Mucor, Helminthosporium, and Alternaria projecting away from agar surfaces in oils often were longest near the glass walls of test-tubes, and many bacterial colonies grew 1 to 5 mm. along the glass away from the agar, prob-

[3] Oil 6 is Standard Oil Co., Diamond Paraffin Oil. Oil 7 is Standard Oil Co., Atlantic Red Oil. Both oils had high percentages of sulphonatable residues.

ably because some oxygen adhered to the glass when the oils were poured into the tubes. Also, oxygen may enter test tubes more rapidly along the oil-glass interfaces than directly through the oils.

J. R. Green, Assistant Chemist for the Montana Agricultural Experiment Station, authorized the use of his evidence of free oxygen in the oils. He placed layers of petroleum oils 3 cm. thick over an aqueous, hyaline solution of KCNS and $FeSO_4$. Oils 21, 15, and 5 turned the test solution red within 1 hour, while Oils 4, 13, 3, 22, 12, 16, 20, 24, and 28 slowly turned the test solution red within 2.5 to 236 hours, in the order listed. Oil 8 [4] very slowly turned the test solution pink within 2 weeks. This experiment gave evidence that free (dissolved) oxygen occurred in these oils, and that Oils 21, 15, and 5 apparently contained more free oxygen than the other oils.

The amounts of fungous growths in these oils was not correlated with differences in the oxygen contents of the oils as revealed in these tests by J. R. Green. The fungi secured food, water, and probably some oxygen from the agar onto which they were inoculated. Presumably they secured some oxygen from the oils. Toxic chemicals in the oils obscured any differences in fungous growths due to differences in amounts of free oxygen in the oils. Mucor, Achlya, and Aspergillus grew only slightly in Oil 8 [4], which was unfavorable to them because it was 53 per cent sulphonatable.

Correlation Between Growth of Fungi in Oils and Tolerance of Apple Leaves to These Oils

Each oil considered in figure 2 was painted on the Hibernal apple leaves of 3 to 25 limbs (a total of 207 limbs) in 1929, 1930, and 1931. Oils 3, 22, 24, and 31 did not kill leaf tissues within 9 to 22 days, and the bark of the attached twigs remained alive during 85 to 145 days. Oils 4, 18, 20, and 25 were more injurious to apple leaves, but they also were sufficiently innocuous for use as sprays on apple leaves. The other oils killed leaf tissues within 1 to 10 days and killed the bark of many of the attached twigs within 39 to 145 days. Oils 11, 14, 19, and 28 were the most injurious. The oils that killed parts of leaves within 1 to 10 days were too injurious for sprays on apple leaves.

Oil 16 was more injurious than its sulphonatable residue (10 per cent) indicated, probably because some of its sulphonatable chemicals were very injurious to the apple leaves and most of the fungi tested.

Tests with fungi made predictable the toxic effects of most of the oils on apple leaves because apple leaves were least injured by the oils in which the fungi grew best (Fig. 2).

[4] Oil 8 is Red Engine Oil, Standard Oil Co.; 53 per cent sulphonatable; viscosity of 410 sec., Saybolt.

Survival of Fungi on Agar in Oils

Reisolations onto agar plates of fungi growing on agar under oils gave the following data on survival: (a) *Helminthosporium sativum* survived for 318 days in 2 cultures under Oil 22, and in 1 culture under Oil 5; 227 days in 2 cultures under Oil 24; 236 days under Oil 3; and 83 days under Oil 31. (b) *Rhizopus nigricans* survived 227 days under Oil 3. (c) *Mucor glomerula* survived during 94 days under Oil 12 and 43 days under Oil 13. (d) *Aspergillus* sp. survived 41 and 83 days in 2 cultures under Oil 3, 83 days under Oil 18, and 83 days under Oil 21. Fifteen of the 29 trials failed to reisolate fungi grown under oils.

The oils were poured from 320 test-tube cultures of organisms after they had been under oils during 72 days. They were in one series in which all of the oils concerned (Fig. 2) were employed. Their test tubes were stored upside down to facilitate draining the oils from the cultures. Examination 9 days later showed that new growths of the organisms had occurred in 95 of the tubes, although much oil still was on the agar. The following figures indicate the survivals of the organisms tested: 14 cultures of *Helminthosporium sativum*, 10 cultures of *Alternaria tenuis*, 4 of *Rhizopus nigricans*, 9 of *Mucor glomerula*, 6 of *Fusicladium alopecuri*, 18 of *Fusarium* sp., Culture 113, 5 of *Fusarium* sp., Culture 35, and 29 cultures of *Achromobacter* sp. Seven cultures of *Achromobacter* sp. in the toxic Oils 2, 5, 12, and 19 grew only after the oils were poured from the cultures.

Only 109 of the 349 cultures tested evidently survived the long periods of immersion in the oils described. Nearly all of the organisms that survived were growing on agar slants instead of the small, thin blocks of agar.

The oils less than 11 per cent sulphonatable were valuable preservatives. Most of the organisms grown under such oils, including the fungi shown in figure 1, A and C, made most of their growths within the first week after inoculation and did not change their appearances during the next 3 years. White oils poured on mature cultures of organisms (including Penicillium) on agar preserved their appearance well. Hyaline, unsulphonatable oils may become useful in differentiating physiological forms of fungi and bacteria.

Effects of Oils on Flowing Protoplasm

Staminal hairs of *Zebrina pendula* Schn. and *Tradescantia fluminensis* Vell. on microscope slides were mounted in Oil 3 saturated with Sudan III and in Oil 24, separate lots of which were saturated with Sudan III and with Oil Red O (Young, 12). Protoplasm was observed to flow in the staminal hairs of Tradescantia 7.8 to 8.3 hours after immersion in the red-stained Oils 3 and 24 on 4 slides and 3 to 3.3 hours after immersion in these oils on 2 other

slides. Protoplasm was observed to flow in the staminal hairs of Zebrina for 4.25 to 6 hours after immersion in Oils 3 and 24 saturated with Sudan III on 3 slides. Later observations on these 9 slides showed no movement in the protoplasm of staminal hairs. Spheres of gas and irregular masses that probably were water appeared near the staminal-hair cells in the oils. Distortions indicating death of these cells appeared within 10 to 24 hours after their immersion in the oils. These observations on protoplasm flowing in staminal-hair cells after immersion in oils showed that these oils were only slightly toxic to the cells within 3 to 8.3 hours.

SUMMARY

Fungi and apple leaves usually were similar in their tolerance for petroleum oils that were less than 11 per cent sulphonatable, and in their intolerance for more sulphonatable oils. Sulphonatable chemicals differed in the ways in which they affected the different organisms.

Fungi can be used to predict the toxic effects of oils on apple leaves by using the following test: (a) inoculating agar slants in test-tubes with *Rhizopus nigricans;* (b) before evident growth occurs, submerging the agar in each tube in an autoclaved oil to be tested; (c) using Nujol or a similar unsulphonatable oil in some tubes and keeping some other tubes without oil for comparisons; and (d) incubating the cultures at 20 to 25° C. for 5 days and recording the height, sporulation, and extent of spread of the hyphae. (e) Only the oils permitting luxuriant growth of Rhizopus probably will be sufficiently innocuous for practical use in sprays on apple leaves.

Fungi growing under oils secured food, water, and some oxygen from the agar. Dissolved oxygen in the oils probably supplied part of the oxygen used by the long hyphae of fungi growing in oils.

The chromogenic bacteria did not produce their pigments under the oils, probably because too little oxygen was available. Most of the purple, red, and yellow colors disappeared from inocula after immersion in the oils for a week.

Protoplasm was flowing in staminal hairs of Zebrina and Tradescantia from 3 to 8.3 hours after immersion in the red-stained oils; this showed the tolerance of the staminal hairs to these oils.

Sheaths occurred on many of the hyphae of Rhizopus and Mucor in oils.

Fungi were reisolated from cultures submerged in oils for 227 to 318 days.

Hyaline, nearly unsulphonatable petroleum oils are useful in preserving the appearances of many kinds of organisms in cultures.

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CHANGES IN PLANT-FOOD INTAKE CAUSED BY A POPULATION OF HETERODERA MARIONI (CORNU) GOODEY ON ANANAS COMOSUS [1]

O. C. Magistad and Juliette M. Oliveira

(Accepted for publication May 10, 1933)

It is believed that nematodes cause damage to crops heavily infested by them mainly because they injure the root system and diminish its absorptive ability. Thus, Vosbury and Winston (7) state, "These nematodes (*Heterodera radicicola*) attack the pineapple plant by penetrating and destroying its fine feeding roots, thus gradually depriving the plant of its means of taking up food and water until, when all the feeding roots are killed, the plant dies." Goodey (3) states that "*Heterodera radicicola* is a misnomer and advocates the use of *Heterodera marioni* (Cornu) instead. Krüger (4), in discussing the effects of *Heterodera schachtii* on sugar beets, comes to the conclusion that nematodes do not damage the absorption ability of the root system but damage the beets by depriving them of their nutriment. Again, Neuwirth (6) records observations on sugar beets confirming the work of Krüger, and states that nematode-infested beets usually exhibit signs of potash deficiency. The good growth of potatoes when moderately infested with *Heterodera schachtii* led Morgan and Peters (5) to consider that "it would certainly appear that the presence of eelworm becomes unimportant when due attention is paid to the proper husbanding of the plants." The present paper describes a test instituted to determine whether or not a population of *Heterodera marioni* on pineapples reduced the absorption of nitrogen from the soil.

EXPERIMENTAL

Sixty wooden tubs of 1220 cu. in. capacity and 11 in. depth were filled with a mixture of field soil and compost. The tubs and contents were steam-sterilized for 8 hours at 15 lbs. pressure per sq. in. Pineapple slips of the Smooth Cayenne variety were planted, one to each pot, on March 22, 1932. At the time of planting, 30 tubs were inoculated with 4 pieces of fresh English Pea root, containing galls of *Heterodera marioni*. These roots were placed about an inch below the butt of the pineapple slip. It is estimated that each tub received a charge of inoculum of 16 to 30 galls each containing 1 to 8 egg masses of about 500 individual eggs.

The pots were placed outside and they were watered at regular intervals. By June 13, 12 weeks after planting, it was very evident that the 20 plants

[1] Published with the approval of the Director as Technical Paper No. 34 of the Experiment Station of the Association of Hawaiian Pineapple Canners, University of Hawaii.

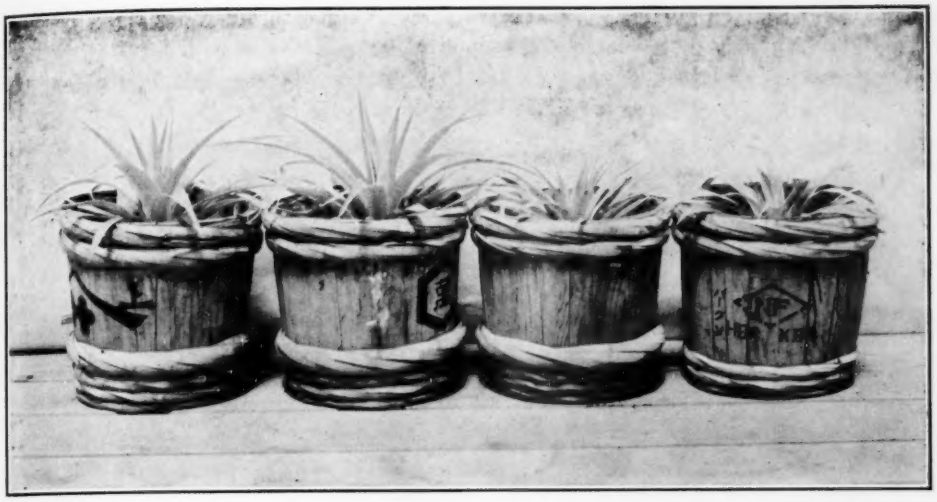

FIG. 1. Comparative growth of pineapple plants with and without inoculation with *Heterodera marioni*. Two pots at left, noninoculated. Two at right, inoculated. Photographed by G. H. Godfrey on June 15.

FIG. 2. Same pots and same arrangement as in figure 1. Photographed by C. H. Spiegelberg on September 20.

inoculated with nematodes were severely retarded. Their appearance at this time is shown in figure 1.

At this time a measure of the amount of growth of each plant was attempted in the following manner: Let us refer to figure 3, showing leaves from noninoculated plants on the left and inoculated plants on the right. The distance on each from the hardened constricted line near the top to the exact tip is the length of this leaf at the time of planting. The remainder of the distance is that of growth since planting. Four leaves of each plant were selected in which the old growth was about 8 to 10 centimeters and the amount of old and new growth measured. The final results on these leaves were:

	Noninoculated	*Inoculated*
Total length	28.81 ± 0.44 cm.	17.11 ± 0.21 cm.
Old growth	8.80 ± 0.04 "	8.90 ± 0.16 "
New growth	20.05 ± 0.44 "	8.20 ± 0.26 "

Representative leaves of the 2 sets with lengths very near the mean of each series are shown in figure 3.

Fertilizer was applied to the lower leaf axils and to the soil adjoining the plants on June 13 at the rate of 2.2 gm. of nitrogen, 0.7 gm. of P_2O_5 and 7.0 gm. of K_2O per tub. The carriers were ammonium sulphate, superphosphate, and potassium sulphate.

GROWTH AND DEGREE OF INFESTATION

On September 19, pictures of the same pots as shown in figure 1 were again taken. These are shown in figure 2, and, when compared with figure 1, show the growth taking place between June 13 and September 19.

On September 20, the plants were dug out and root examinations and plant weights were obtained. The procedure was similar to that of Collins and Hagan (2), except that the roots were clipped from the butt, weighed, and examined separately.

The noninoculated plants were not entirely free from galls, some slight infection having come from the planting material or tools at the time of planting. Thus in Lot A, there were five plants having galls, the numbers on each being 5, 26, 1 and 13, respectively. In Lot B, two plants had galls, the number being 2 and 4, respectively.

The number of galls on the inoculated plants was great for the age of the plant. Every plant was infected. A considerable number of the roots, however, had no galls. The majority of the large galls were concentrated on the shorter roots.

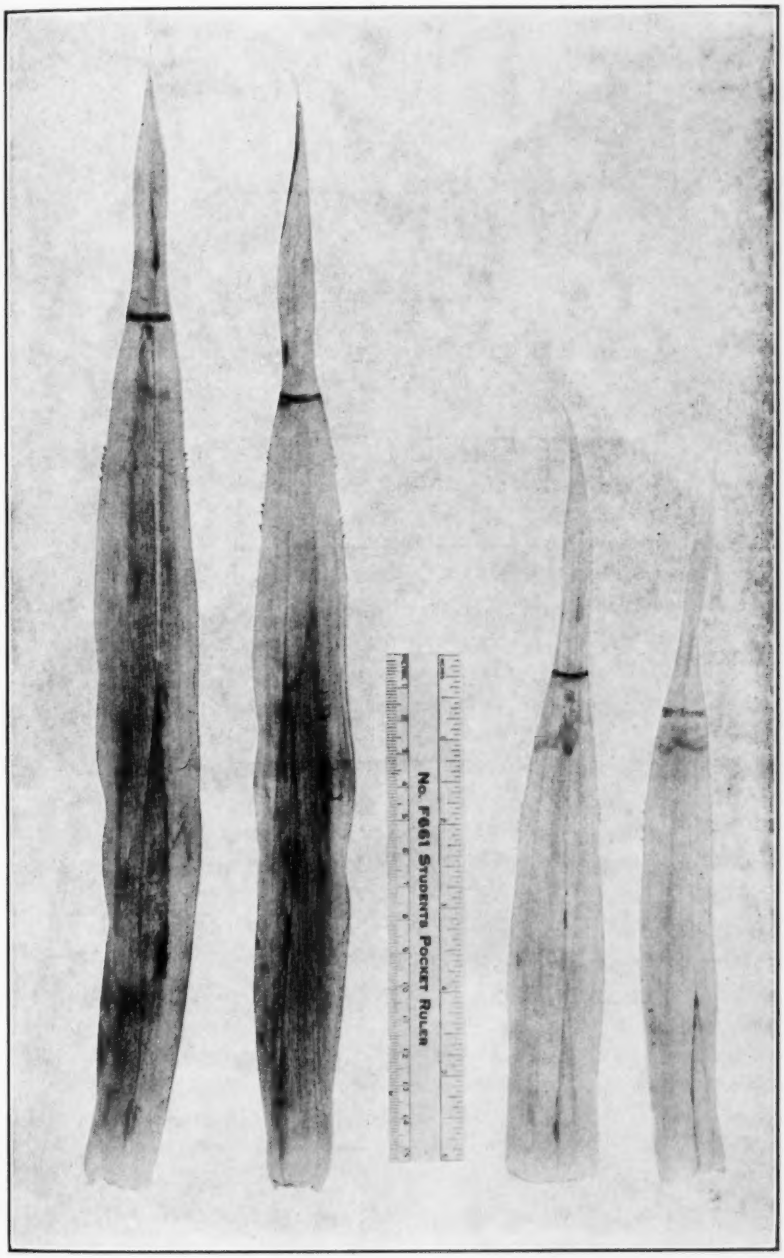

FIG. 3. Comparative growth of pineapple leaves from plants with and without inoculation with *Heterodera marioni*. Leaves at left, mean of noninoculated plants. Leaves at right, mean of inoculated plants. The portion of each leaf above the transverse mark grew before planting and that below after planting.

TABLE 1.—*Root number, length, weight, and number of galls per plant*

Lot	Av. root wt. in grams	Av. number of roots	Av. total length cm.	Length average root cm.	Av. number galls per plant		
					Main roots	Lateral roots	Total
A[a]	40.49 ± 1.97	96.80 ± 2.75	1538.27 ± 70.47	15.89	1.40	1.60	3.00
B	35.95 ± 1.81	88.87 ± 2.81	1483.73 ± 64.05	16.66	0.47	.....	0.47
C	32.93 ± 2.33	60.00 ± 2.65	646.53 ± 39.24	10.77	36.87	60.00	96.87 ± 11.6
D	24.62 ± 1.48	57.27 ± 2.49	681.93 ± 24.78	11.91	32.33	43.27	75.60 ± 14.7

[a] Treatment—A—Noninoculated, unfertilized. B—Noninoculated, fertilized. C—Inoculated, unfertilized. D—Inoculated, fertilized.

The number of galls on fertilized versus nonfertilized inoculated plants seems to indicate that fertilizer, for some reason or other, has slightly retarded the entry of nematodes into the roots. The difference in galls per plant (C—D) is 21.27 ± 18.7.

As regards root number, fertilizers have caused a slight reduction. The value of A minus B equals 7.93 ± 3.95 and C minus D equals 2.83 ± 3.64; both lack significance. Nematodes have caused a greater decrease in root numbers, the differences being:

$$A - C = 36.80 \pm 3.82$$
$$B - D = 31.60 \pm 3.75$$

Both of these decreases are very highly significant. These results are opposed to those of Collins and Hagan (2) who found no evidence that nematodes caused a decrease in root number.

In our experiment, nematodes have decreased the average total length of roots per plant with overwhelming odds.

The average percentage growth based on initial weight for the period March 22 to September 20 was: Lot A—noninoculated, nonfertilized, 614.2 ± 15.2; lot B—noninoculated, fertilized, 695.9 ± 21.6; lot C—inoculated, nonfertilized, 401.0 ± 19.5; lot D—inoculated, fertilized, 420.0 ± 66.3.

A calculation to determine whether or not the difference between treatments A and C is significant gives very great odds, likewise for the difference B—D. This indicates that in this experiment nematodes did retard growth.

A slight but nonsignificant increase in growth was caused by fertilization of inoculated plants. This appears to contradict Krüger's theory that nematodes rob the plant of nutriment but do not prevent its entrance.

NITROGEN ABSORPTION

Six plants from each of the 4 combinations of treatments were composited. The percentages of total nitrogen were determined in these composite samples by the Gunning method modified to include the nitrogen of nitrates (1), and also in a sample of the original planting material. The nitrogen contents of the various lots were:

Lot A—noninoculated, nonfertilized, 1.256 ± 0.002; lot B—noninoculated, fertilized, 1.468 ± 0.002; lot C—inoculated, nonfertilized, 1.203 ± 0.002; lot D—inoculated, fertilized, 1.407 ± 0.004.

The data above clearly indicate that fertilization has increased the nitrogen content of these plants. This effect of nitrogen fertilizers to increase the percentage of nitrogen content of pineapple plants has also been noted in a number of field experiments.

The nitrogen contents of the various lots on September 20, together with weights of the final plants, original slips, and nitrogen contents of the original slips, permit us to draw up a table showing the amount of nitrogen taken up by each plant between March 22 and September 20.

The values are as follows:

Lot A—noninoculated, nonfertilized, 1.65 ± 0.058; lot B—noninoculated, fertilized, 2.15 ± 0.087; lot C—inoculated, nonfertilized, 0.89 ± 0.032; lot D—inoculated, fertilized, 1.13 ± 0.055.

We first notice that plants in A were able to obtain 1.65 grams nitrogen per plant from the soil alone. The compost in this soil is undoubtedly responsible for this large liberation of nitrogen. The difference B – A is 0.50 ± 0.104. The odds here are exceedingly great that in another experiment similarly conducted A would not equal or exceed B.

The amount of fertilizer added was 2.2 grams nitrogen per plant. If we consider that 1.65 grams were furnished by the soil, then only 0.50 gm. came from the fertilizer in B, or a utilization of 23 per cent. Heavy rains may have leached a considerable quantity of nitrogen from the soy tubs and probably prevented further absorption.

The addition of fertilizer to nematode-infested plants has increased the quantity of nitrogen assimilated per plant by 0.24 ± 0.066 gm. This difference is significant with odds of 63 to 1. The data indicate that the application of fertilizer to plants infested with nematodes has significantly increased the weight and percentage of their nitrogen content, but fail to demonstrate the resultant growth (increase in dry matter).

The actual decrease in weight and percentage decrease in total nitrogen intake caused by nematodes were as follows:

Lot A – C, 0.76 ± 0.067, 46.1 ± 4.4; Lot B – D, 1.02 ± 0.103, 47.5 ± 5.3.

The above values show that under the conditions of this experiment the presence of nematodes has caused a decreased nitrogen intake of 40 to 50 per cent.

SUMMARY

Pineapple planting material was placed in sterile soil to which, in 30 pots out of 60, an inoculum of *Heterodera marioni* had been added. Twelve weeks after planting, 15 pots of each set were fertilized and at the end of 6 months plant examinations and analyses were made. The data accumulated support the following statements as being significant, odds 30:1 or more, for this experiment:

Nematodes have decreased the average total length of roots per plant as well as the number of roots.

Nematodes have reduced the rate of growth of the plant, as judged by dry-matter contents.

Fertilizers seem to have increased the rate of growth of noninfested plants, odds 25:1 but not that of the infested plants.

The presence of nematodes retarded the absorption of nitrogen by 40 to 50 per cent.

Application of fertilizer has markedly increased the percentage of nitrogen in the plant but has resulted in increased growth only in the case of noninfested plants.

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AN AUTOMATIC MOISTURE-RECORDING DEVICE

ROGER WINTERS AND CYRIL G. SMALL

(Accepted for publication June 1, 1933)

The duration of periods of wetting by rain or dew is often an important factor governing infection under field conditions. In experimental work it is desirable to know the length of these periods and when they occur, but it is obviously impractical to make observations frequently enough to determine the exact lengths of probable infection periods throughout an entire season. It is, therefore, desirable to have an automatic device to record the length of time the leaves remain wet.

Many organisms require the continuous presence of a film of water for several hours before they cause infection. In developing the device described below, it was, therefore, decided that it should be operated by the film of moisture on the surface of the leaf. Since this film could not provide the energy needed to operate a recording device, it was decided to use the film to control an electrical circuit from which the necessary energy could be obtained. However, if an electric current were passed through the water film, electrolysis would result, and the products thus formed might injure the leaf. It was, therefore, evident that the recorder must be operated by voltage and not by current. This requirement was met by using a vacuum tube.

A semipictorial diagram of the electrical circuit appears in figure 1, A. A type '45 radio tube is supplied with voltage from a simple "B eliminator," which uses a type '80 rectifier. Sufficient filtration of the direct current is obtained with a single 8-microfarad condenser, C. A 10,000-ohm resistor, R_1, in the negative plate-return lead furnishes a grid bias voltage for the '45 tube. The negative end of this resistor is connected to earth. The grid lead of the '45 tube is connected to a small metal plate, M, which is separated from the leaf of a plant by means of a thin insulator, I. When the leaf becomes wet the water film bridges across this insulator, making electrical contact between the metal plate and the leaf. Since the leaf is grounded through the plant, the negative bias voltage developed across R_1 is applied to the grid of the tube, causing a large decrease in the plate current. When the leaf dries, the grid circuit is opened, and the plate current increases. In addition to providing the biasing voltage for the '45 tube, the resistor R_1 also prevents the current in the plate circuit from increasing to a point where it might damage the tube or other parts of the apparatus when the grid voltage is removed from the tube. The resistor R_2 is a "bleeder" across B + and ground.

In the plate circuit of the '45 tube is an electromagnet. The armature of this magnet is connected to a pen that rests on a moving drum. The

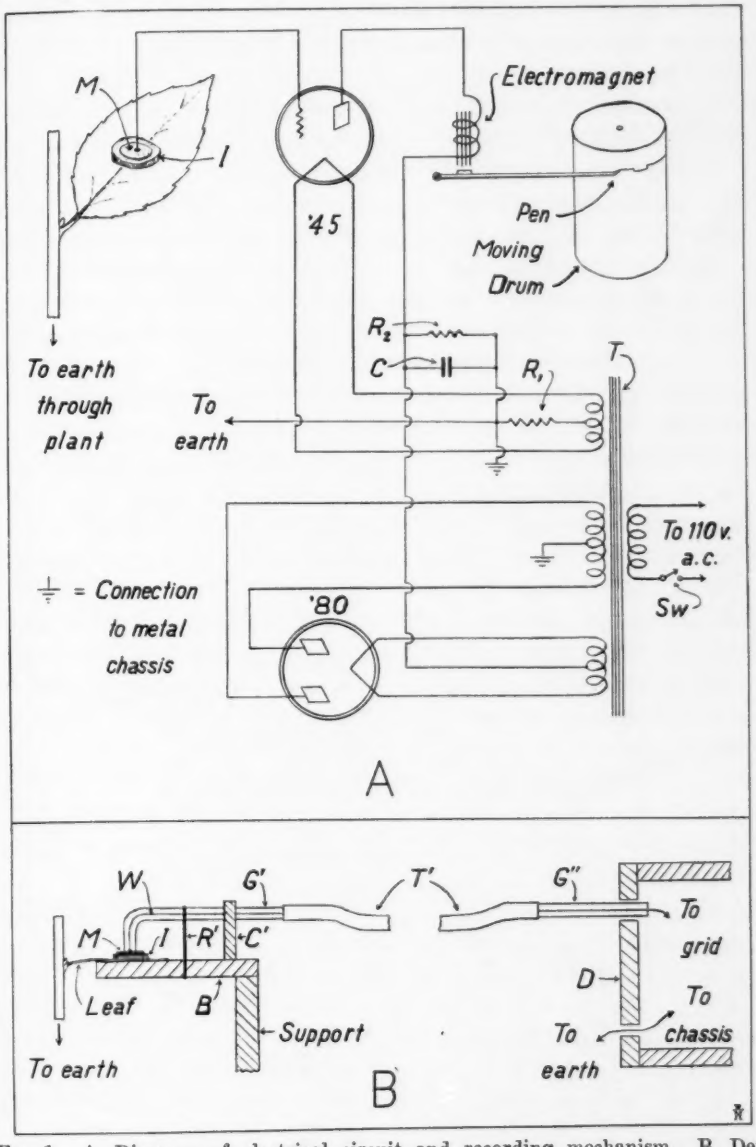

FIG. 1. A. Diagram of electrical circuit and recording mechanism. B. Detail of connection between grid of vacuum tube and leaf.

change in plate current of the tube causes a movement of the armature of the electromagnet and consequently of the pen. Therefore a record, which

may be interpreted in terms of wetness or dryness of the leaf, is made on the drum.

In order to obtain sufficient power to operate the recording pen, it is necessary to use a tube with a fairly large plate current. The '45 proved most satisfactory, although a '71-A has also been tried, with about the same degree of success. The electromagnet used is actually a Western Electric type R-1314 telephone relay, with the relay contacts bent up out of the way. If one wished to operate some device other than a recording pen, it would be quite feasible to use the contacts of the relay for opening and closing appropriate electrical circuits. In this way lights, bells, motors, or other electrical equipment could be controlled. For such purposes, the moisture apparatus could be operated from batteries, using either a 201-A or 199 tube, provided that one employed a relay that was sufficiently sensitive to operate on the smaller plate currents of these tubes.

The pen is attached to the armature of the relay and the latter mounted in the case of a recording thermograph, which originally contains a revolving drum operated by clockwork, and another pen that records the temperature on this drum. The moisture-recording pen is placed near the top of the drum in order not to interfere with the temperature-recording pen. Thus both records are made on the same chart. If a recording thermograph were not available, it should be possible for anyone with a little mechanical ingenuity to devise a suitable substitute for the moving drum. In many cases, however, the thermograph is part of the field equipment and represents no extra expenditure. As mentioned above, the relay contacts are bent up out of the way. This is done to allow the armature to move more freely and to obtain a greater movement of the recording pen. A small coil spring is added to pull the armature away from the magnet when the plate current decreases. Such a spring can be made from fine piano wire, or a small weight might be fastened to the armature to obtain the same effect.

All of the parts except the relay and the metal plate, M, are mounted on a metal chassis. This may be made from a sheet of heavy galvanized iron by bending its edges down at right angles to provide about $1\frac{1}{2}$ inches of space beneath for the wiring and for mounting the tube sockets and resistors. There should be two $1\frac{1}{8}$ inch holes in the chassis for mounting the tube sockets and such other holes as are required for the wiring and for mounting the transformer, condenser, and resistors. The transformer and condenser should be mounted on top of the chassis and should be located so that the wiring will be as simple and direct as possible. All electrical connections should be well soldered, using rosin flux. Acid flux must *never* be used for electrical work, since electrolytic effects are set up when current flows and the joint is quickly destroyed.

The metal plate, M, may be of thin copper or brass or heavy tinfoil. It is soldered to a wire that connects it to the grid of the '45 tube. This wire should be enclosed in either glass or rubber tubing, sealed moisture-tight at each end with paraffin. It is *absolutely imperative* that this wire be kept dry and thoroughly insulated from the earth (and metal chassis), as even a high resistance contact between grid and earth is sufficient to cause a decrease in plate current and, therefore, to operate the recording pen.

The insulator, I, may be a thin piece of glass, such as is used for a microscope cover slip, or a small piece of mica, rubber, or other good insulator of sufficient thinness that a film of water can creep over it and make electrical contact between metal plate and leaf. The thickness and nature of the insulator determine to some extent the sensitiveness of the device. Thin glass insulators may even cause the device to operate in a very humid atmosphere, apparently because the glass condenses a film of moisture on its surface. A fairly thick rubber insulator ($\frac{1}{8}$ to $\frac{1}{4}$ inch) will usually prove more satisfactory. The insulator may be fastened to the metal plate by means of shellac, but care must be taken that the top of the metal plate be not insulated with a coating of shellac. In very moist localities, such as those near the seacoast, the device may prove too sensitive for satisfactory operation unless special precautions are taken to protect all parts against the extreme humidity.

Figure 1, B, shows the method by which the connection between leaf and grid is made in the field. One end of a strip of wood, B, about $\frac{3}{4}$ inch wide is attached to some suitable support so that the free end is placed where a leaf may be clamped to it without injury to the leaf. It is probably best to clamp the leaf in a horizontal position. The wire, W, with the metal plate, M, attached, is passed through a piece of glass tubing, G′, and the metal plate brought into contact with the end of the glass. The metal plate is then sealed to the glass tubing by means of paraffin, care being taken not to insulate the entire upper surface of the metal with paraffin. This tube, with the metal plate and insulator attached, is held in position by a wooden clamp, C′, so that the insulator rests upon the leaf. A rubber band, R′, holds it firmly against the leaf and effectively clamps the leaf between the insulator and the wood strip, B.

Tube G′ is connected to a second piece of glass tubing, G″, by means of a piece of rubber tubing, T′. The entire outside surface of the glass and rubber tubing is coated with paraffin and the end of tube G″ is sealed with the same material. Tube G″ should carry the wire through the case that houses the electrical apparatus. The wire should be connected directly to the grid contact of the tube socket, but care must be taken not to allow the wire to touch any of the other metal parts of the apparatus.

It would be satisfactory to pass the wire through one long piece of glass tubing, sealing the ends with paraffin, but such an arrangement is fragile and lacks flexibility. The rubber tubing furnishes this flexibility, while the glass tubing at either end furnishes rigidity for clamping.

For use in the field, the apparatus is housed in a small weather-tight house, D, patterned after the government weather stations. An upper shelf holds the metal chassis, while a lower shelf, having ventilating shutters at the sides, accommodates the thermograph and relay. The house is located close to a plant of the species being used for experiment and the connection made to a leaf. The metal chassis is connected to earth by means of a wire, thus completing the circuit through the plant. The location must also be close to an available source of 110-volt alternating current.

The parts required for construction of the moisture-recording device are as follows:

Two UX-type (four-prong) "wafer" tube sockets; 1 type '45 radio tube; 1 type '80 radio tube; 1 resistor, 10,000 ohm, 25 watt (R_1); 1 resistor, 25-000 ohm, 25 watt (R_2); 1 a.c. switch (Sw); 1 dry electrolytic condenser, 8 microfarad, 450 volt (C); 1 power transformer (T) with the following windings: (a) 110 volt primary; (b) $2\frac{1}{2}$ volt, $1\frac{1}{2}$ ampere secondary, center tapped, for '45 tube; (c) 5 volt, 2 ampere secondary, center tapped, for '80 tube; (d) high voltage secondary, center tapped, supplying 250 to 300 volts each side of center. One relay, Western Electric type R-1314; 1 recording thermograph, or substitute for moving drum; 1 metal chassis; 25 feet of "push-back" insulated wire; rosin-core solder; bolts and nuts; glass and rubber tubing, etc.

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ONION-BULB DECAY CAUSED BY ASPERGILLUS ALLIACEUS[1]

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INTRODUCTION

Aspergillus alliaceus Thom and Church is a yellow-spore, sclerotium-forming species, that has been intercepted twice on garlic imported into this country from Italy. In the first case it was found in a shipment arriving in New York City in 1919, and in the second instance in a shipment that arrived at the port of Charleston, South Carolina, in 1929. The fungus was described by Thom and Church[2] on the basis of a culture isolated from the first collection. They reported another strain, similar in morphology, which was obtained from a dead blister beetle. As already pointed out,[3] this fungus was shown to be an aggressive decay-producing parasite when inoculated through wounds into mature bulbs of onions and garlic. It has never been reported in nature upon onion, but since it has been introduced into this country with garlic shipments it became important to study its potentialities as the cause of an onion disease.

THE CAUSAL ORGANISM

Thom and Church[2] give a detailed description of the organism, including its cultural characters on Czapek's solution agar. On this substrate white aerial mycelium spreads rapidly over the surface, becoming less pronounced as the sclerotia are produced. Conidiophores are produced bearing heads, yellow at first, but becoming brown with age. The sclerotia are white at first, turning brown and finally black. The darkening begins at the end of the sclerotium in contact with the substrate. The upper end remains white for a time, and at this stage it usually bears a colorless, glistening drop of liquid that disappears as the entire sclerotium becomes black. Growth on potato-dextrose agar is quite similar, except that the aerial mycelium in the early stages is more profuse.

The growth on potato-dextrose agar in Petri dishes was studied over a range of constant temperatures. Growth was meager at 15° C. and below. The rate of radial expansion increased directly with the temperature up to an optimum at about 32° C. Growth was greatly retarded at 40° C. and

[1] Cooperative investigations between Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture and Department of Plant Pathology, University of Wisconsin.

[2] Thom, C., and M. B. Church. The Aspergilli. 272 p. Williams & Wilkins Co., Baltimore. 1926.

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the maximum temperature is somewhat above that point. The South Carolina isolate produced conidia more abundantly at most temperatures, while the New York isolate produced sclerotia in greater numbers (Fig. 1). Sclerotia developed most promptly at 32°, and progressively less rapidly at higher and lower temperatures, except at 40°, where none occurred. At 20° and below they were somewhat larger than at higher temperatures. Conidiophores were produced most abundantly at 40°, where they were closely crowded together. At 32° they were numerous but more widely scattered. At lower temperatures they were relatively sparse.

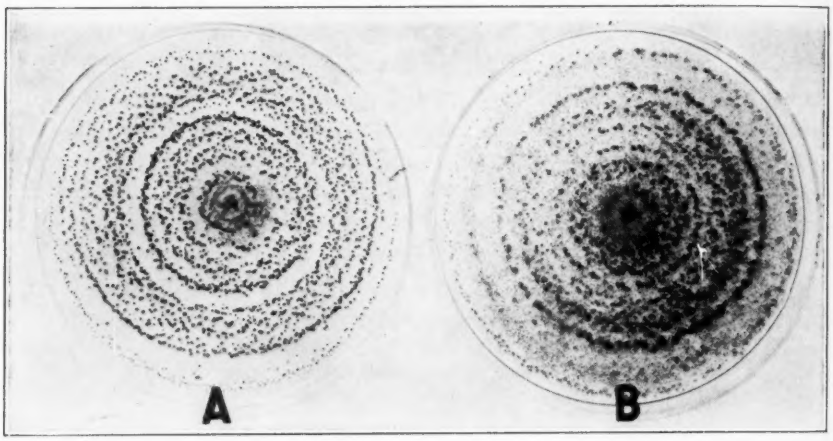

FIG. 1. Petri-dish cultures of *Aspergillus alliaceus* on potato-dextrose agar, 9 days after inoculation at 32° C. A. New York isolate. B. South Carolina isolate. Note greater conidial production in B.

PATHOGENICITY

The pathogenicity of the fungus on mature onion bulbs and on garlic has been demonstrated repeatedly by inoculating them through wounds and placing them in moist chambers or in glassine bags. The first sign of the disease is a slight swelling and darkening of the tissue around the point of inoculation. The bulb tissue becomes watersoaked and softens and individual cells are affected slightly in advance of the hyphae. A distinct line of demarcation occurs between the advancing lesion and the unaffected healthy tissue. The older decayed tissue shrinks and becomes brownish. At favorable temperatures a dense, white mycelium mat forms between the bulb scales. Sclerotia are formed, first around the point of inoculation and later throughout the decayed tissue. They are white at first, turning black as they mature. Conidiophores bearing conidia, yellow *en masse,* are borne in abundance on the surface of the decaying bulb at high relative

humidity. Premature sprouting of affected bulbs is often stimulated. The decaying tissue desiccates readily, assuming a dry, spongy texture.

Inoculated onion bulbs were placed in glassine bags and incubated at constant temperatures varying from 8° to 44° C. After 21 days the bulbs at 36° C. were completely rotted and progress of the disease was nearly as rapid at 28° and 32°. At 20°, 24°, and 40° there was very slight decay, while at 16° C. none developed during a period of three months.

The possibility of the fungus becoming a parasite of growing onion plants was investigated. Cultures on a mixture of sterilized corn-meal and sand were allowed to grow until sclerotia had formed in abundance. They were mixed thoroughly with greenhouse compost soil, which was placed in 5 containers and inserted in soil-temperature-control tanks, one each at 16°, 19°, 22°, 26°, and 30° C. Four White Portugal onion sets were planted in each container and allowed to grow for 83 days. The moisture content of the soil was held at approximately 60 per cent of the water-holding capacity for 34 days and at 80 per cent for the remainder of the period. No signs of disease development occurred on any of the plants at any temperature during the course of the experiment. Two tanks were set up at 28° C. containing 12 containers of inoculated soil and 4 containers of noninoculated soil. Ten White Portugal onion sets were planted in each container. In one-third of the containers the sets were each wounded with a needle before planting. Fifteen days later the plants in another third of the containers of inoculated soil were wounded near the bases of the scales and mycelium from a pure culture injected into the wound. When the plants were all pulled and examined 53 days after the experiment was started, no signs of disease were noted in any of them. It is apparent that the fungus does not attack growing onion plants.

DISCUSSION

The organism has been found twice causing a decay of imported garlic bulbs. It also is pathogenic on onion bulbs, although it has not been reported upon the latter in nature. Compared with other decay-producing parasites of onion bulbs, *Aspergillus alliaceus* is a high-temperature organism, its optimum activity being within the range of 28° to 36° C. This fact probably limits its potential danger as the cause of a serious disease in this country, since it can be effectively checked at storage temperatures below 20° C. It does not attack the growing onion plant and appears to be confined in its parasitic activity to the mature bulbs.

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VARIETY TESTS IN THE DIFFERENTIATION OF TWO COTTON WILTS [1]

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Wilt caused by *Fusarium vasinfectum* Atk. is the most destructive disease of the cotton plant in most cotton-growing regions where the even more destructive Phymatotrichum root rot is not present. In Texas, wilt occurs more generally in the sandy-loam soils in the eastern part of the State. It has been shown previously [2] that these soils in which wilt occurs are generally somewhat acid in reaction. Phymatotrichum root rot, on the contrary, is most prevalent in heavy, neutral to basic soils. It was, therefore, a matter of special interest when, in 1927 and 1928, a wilt was found attacking cotton near Waxahachie, Ellis County, Texas, in alkaline, heavy clay soils in which Phymatotrichum root rot also was prevalent.[3] The symptoms of this wilt differed somewhat from those of the typical *Fusarium vasinfectum* wilt. With Fusarium wilt the stem of the cotton plant was discolored mostly in the outer woody tissue, while with the new wilt the discolored area was confined mainly to the interior cylinder of both roots and stems. *F. vasinfectum* was rarely among the fungi isolated from the diseased plants from Waxahachie; however, none of the various other fungi isolated from the plants have yet been proved the cause of the disease. It, therefore, still remained in some doubt whether the new wilt (which for convenience is designated temporarily as Waxahachie wilt) might not be, perhaps, the well-known Fusarium wilt adapted to calcareous soils.

In 1929 a field heavily infested with Fusarium wilt was located near Tabor, Brazos County, and this afforded opportunity to test some of the standard wilt-resistant varieties for resistance to Fusarium wilt as it occurs in Texas. At the same time, parallel plantings of the varieties were made at Waxahachie, to find whether the new wilt resembled Fusarium wilt in its effect on different varieties of cotton. Part of each lot of seed was planted in replicated rows in the field near Tabor, and the remainder of each lot in replicated rows in the field at Waxahachie. Total numbers of plants ranged from 150 to 200 plants of each variety in the 1929 test at Waxahachie to 500

[1] Published with the approval of the Director as Technical Contribution No. 265 of the Texas Agricultural Experiment Station.

[2] Taubenhaus, J. J., Walter N. Ezekiel, and D. T. Killough. Relation of cotton root rot and Fusarium wilt to the acidity and alkalinity of the soil. Texas Agr. Exp. Sta. Bul. 389. 1928.

[3] Taubenhaus, J. J., W. N. Ezekiel, and H. E. Rea. A new cotton wilt. Phytopath. 19: 171–173. 1929.

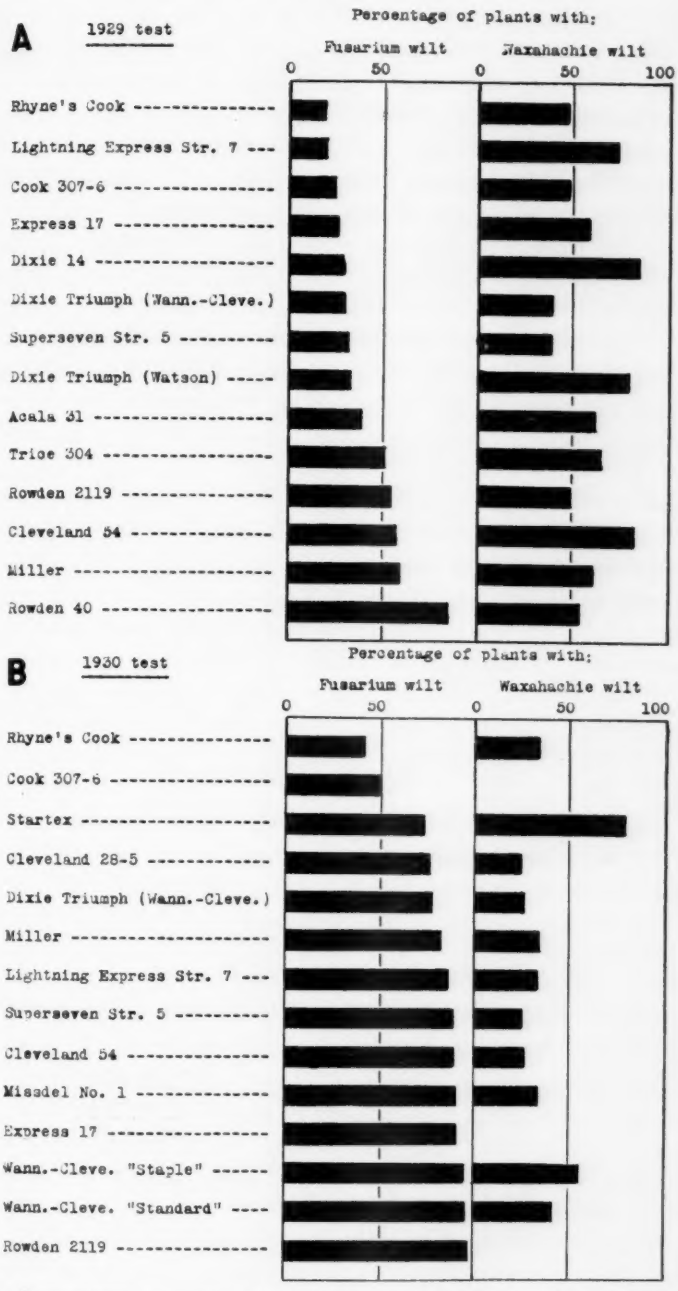

FIG. 1. Percentages of cotton plants killed or with characteristic internal discoloration. A. 1929. B. 1930.

to 700 plants of each variety in the 1930 test at Tabor. At the end of the season each plant was pulled and the stem and root cut open and examined. It was possible thus to determine not only the percentages of plants killed, but also the percentages of living plants that showed internal symptoms of injury.

The results obtained in 1929 and 1930 are shown in figure 1. In these graphs, the total percentages of plants attacked by Fusarium wilt in the tests in Brazos County are shown on the left, and those of plants attacked by Waxahachie wilt in the tests in Ellis County on the right. Quite obviously, the varieties did not react in a similar manner to these 2 diseases. For 1929, the 5 varieties most resistant to *Fusarium vasinfectum* wilt were instead 3rd, 11th, 5th, 7th, and 14th in the order of resistance to Waxahachie wilt. The Watson and Wannamaker-Cleveland selections of Dixie Triumph cotton were almost equally resistant to Fusarium wilt, while they reacted quite differently to Waxahachie wilt. Taken as a whole, there was no significant correlation in the reaction of the varieties to the 2 diseases.

Similar results were obtained in 1930 (Fig. 1, B). Conditions were very favorable for the Fusarium wilt in Brazos County and heavy losses occurred in the test plots. As in the preceding year, the variety Rhyne's Cook showed the least wilt infection, and its mean yield and that of Cook 307–6, from which Rhyne's Cook was originally selected, were more than double the yields obtained from the other varieties. Yet, Rhyne's Cook was 7th in resistance to Waxahachie wilt; and Startex was lowest in resistance to this disease, although it had been 3rd in resistance to Fusarium wilt. There was again no significant correlation between the reactions of the varieties to the 2 diseases.

In 1931 and 1932, wilt-resistant varieties were tested against *Fusarium vasinfectum* wilt only in plots at College Station. These additional results with Fusarium wilt, which were also in accord with results of cotton-variety tests in other States, yielded further evidence of the reasonably stable reaction of varieties to this particular disease as shown by percentages of infected plants. Thus Rhyne's Cook led in freedom from infection for 2 years, and was second best for the other 2 years. Cook 307–6 ranked 3rd, 2nd, and 1st, respectively, during the 3 years it was included in the tests. Among the less resistant varieties, Rowden 2119 ranked 11th, 14th, 18th, and 11th again during the 4 years; and Cleveland 54 ranked 12th, 10th, 12th, and 16th. Kasch and Gorham's Lonestar were lowest in wilt resistance in both years that they were tested. Detailed results of these variety tests will be given in a separate paper.

SUMMARY

Cotton varieties have been tested in Brazos County, Texas, for resistance to *Fusarium vasinfectum* wilt, and in Ellis County for resistance to an appar-

ently different wilt found there and designated temporarily as Waxahachie wilt. Varieties resistant to *Fusarium vasinfectum* wilt were generally consistently resistant, while susceptible varieties were fairly consistently susceptible in the various years. Varieties resistant to this disease were not, however, necessarily resistant to the Waxahachie wilt, nor was susceptibility to the one disease correlated significantly with susceptibility to the other. It therefore appears reasonable to conclude that the differences between the reactions of cotton varieties to the Waxahachie wilt and those to known *Fusarium vasinfectum* wilt constitute an additional distinction between the two diseases.

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QUANTITATIVE DETERMINATION OF SULPHUR ON LEAVES BY TITRATION

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(Accepted for publication April 25, 1933)

Numerous quantitative determinations of the amount of sulphur adhering to the foliage of trees treated with sulphur fungicides have been made by the writer during the course of 5 years' experimental work with the dusting and spraying of apples. A gravimetric method of analysis, combining the more desirable features of methods used by Fitch [1] and Streeter [2], was used for most of this work. Because this method involves a long procedure and requires equipment and facilities difficult to obtain in a field laboratory, a new method [3] has been developed, the basis for which was suggested to the writer by R. Winters, of the Department of Plant Pathology, Cornell University.

This new method is based upon the volumetric determination of the amount of sodium hydroxide used when the sulphur is oxidized to sodium sulphate. The sulphur is first heated with a known amount of a strong solution of sodium hydroxide until all of the sulphur has reacted to form soluble sulphides or thiosulphates. These compounds are then oxidized to sodium sulphate by the action of hydrogen peroxide. When the reaction is complete the remaining sodium hydroxide is determined by titration with a standard solution of hydrochloric acid. The amount of sulphur can then be calculated from the amount of sodium hydroxide used in the reaction.

The analytical procedure may be outlined briefly as follows:

1. Collect representative samples of 150 leaves each, placing them directly into glass beakers. If possible, each sample should consist of equal numbers of leaves taken from 3 or more trees. Duplicate samples may be taken in order to check the accuracy of the procedure.
2. Make a separate determination for each sample, as follows:
   a. Pour about 300 cc. carbon tetrachloride into a large beaker. Immerse about 40 leaves in the tetrachloride and let stand for 10 minutes. Turn the leaves over with a glass stirring rod and let stand for another 10

[1] Fitch, H. W. Revised method for the quantitative determination of sulfur fungicides on foliage. Phytopath. **16**: 427–428. 1926.

[2] Thatcher, R. W., and L. R. Streeter. The adherence to foliage of sulfur in fungicidal dusts and sprays. N. Y. (Geneva) State Agr. Expt. Sta. Tech. Bul. 116. 1925.

[3] After this work was completed the writer learned that Fr. Kühl, working at Copenhagen, used the same method for analyzing weighed samples of sulphur. This was published in the following article:

Kühl, Fr. Ein Schnellverfahren zur Gehaltsbestimmung von Schwefel. Ztschr. Analyt. Chem. **65**: 185–186. 1924.

minutes. Remove the leaves a few at a time, pulling them out by the stems, and rinse in a second beaker containing about 250 cc. of carbon tetrachloride. Carefully shake off all droplets of the solvent and discard the leaves. Immerse successive 40-leaf portions of the sample in the first beaker and repeat the washing until all of the sample has been treated.

b. Filter the contents of both beakers through a thin tuft of absorbent cotton and place the filtrate in a 500-cc. florentine flask. Rinse all beakers and the filter with clean carbon tetrachloride and add the rinsings to the flask.

c. Place the flask on a hot plate and distill until the volume of the sample has been reduced to about 10 cc. Save the distilled solvent for future use.

d. Heat the sample on a steam bath until *all* of the solvent has been removed.

e. Add 5 cc. of distilled water and make neutral to methyl red indicator, using approximately 0.1 normal solutions of either hydrochloric acid or sodium hydroxide.

f. Using a pipette, add 20 cc. or more[4] of a 2.0 normal solution of sodium hydroxide, close the flask with a "protection tube"[5] (Fig. 1), and heat on the steam bath until there is no free sulphur remaining in the flask.

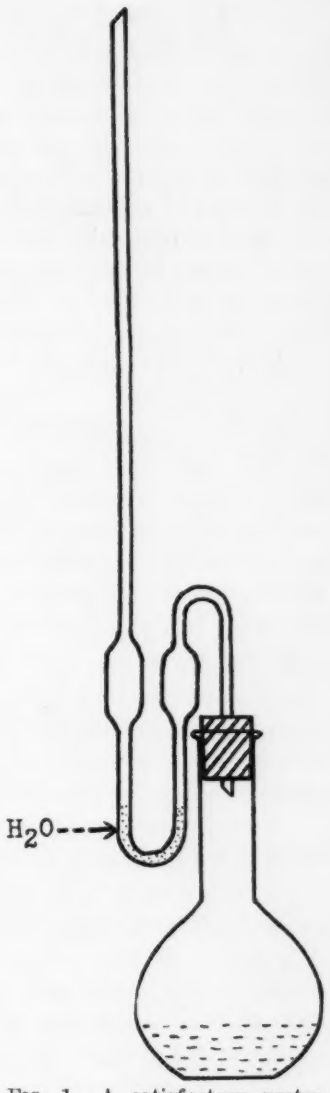

FIG. 1.—A satisfactory protection tube. Its use prevents any loss of solution from the flask. $\times \frac{1}{4}$.

g. Allow the sample to cool to room temperature and then slowly add a 30 per cent solution of hydrogen peroxide, using 5 cc. for each 20 cc.

[4] The exact quantity of sodium hydroxide to be added to the flask will depend upon the amount of sulphur expected in the sample, each 0.5 gram of sulphur requiring about 20 cc. of the sodium hydroxide solution. If free sulphur remains in the flask after 5 to

of sodium hydroxide. Heat on the steam bath for 3 to 5 hours, cool, and add a second portion of hydrogen peroxide. Heat again for 1 hour and then allow to cool.

h. Titrate the remaining sodium hydroxide against a standardized 1.0 normal solution of hydrochloric acid, using methyl red indicator.

3. Run a blank determination with each set of samples, using the same amount of sodium hydroxide as was used in the samples. Titrate this blank to determine the exact amount of sodium hydroxide used.
4. Calculate the amount of sulphur present in each sample, as follows:
   a. cc. HCl used to titrate blank – cc. HCl used to titrate sample = cc. HCl equivalent to NaOH used to form Na_2SO_4
   b. cc. HCl equivalent to NaOH used to form Na_2SO_4 × "factor" = grams of sulphur
   c. "factor" = normality of standard HCl solution × 0.016

This method of analysis was tested extensively during the summer of 1932. Samples analyzed by this method were later analyzed gravimetrically in order to compare the 2 methods. Data obtained from 48 comparisons show that when the amount of sulphur in the sample is greater than 0.050 gram the variation in the amount analyzed by the 2 methods is considerably less than the variation found when duplicate leaf samples are analyzed by either method. The amount found by titration is usually slightly greater than that found gravimetrically, averaging 2.9 per cent more. When the amount of sulphur is less than 0.050 gram a higher variation is found between the analyses obtained with the 2 methods. This appears to be due to inaccuracy in the titration method.

Preliminary tests were made in an effort to increase the accuracy of the method with samples containing small amounts of sulphur. A series of duplicate samples, each containing 0.003 gram of sulphur, was analyzed, using 5 different strengths of sodium hydroxide and 4 of hydrochloric acid. The results obtained indicate that the accuracy of the method can be increased by the use of more dilute solutions. The use of 0.5 normal hydroxide and 0.25 normal acid reduced the error to about 10 per cent. The accuracy of the method was still further increased by the use of 0.125 normal

8 hours of heating more sodium hydroxide should be added. Using 150-leaf samples, the writer has found amounts of sulphur varying from slightly over 2.0 grams to as little as 0.003 gram.

[5] The "protection tube" is used to prevent the oxygen that is evolved from carrying a fine mist of solution out of the flask. Protection tubes may be made from large-bore glass tubing, bending it so that gases can enter or leave the flask only after bubbling through a column of distilled water in the tube. The contents of the tube should be washed into the flask just before making the titration. Figure 1 shows a more satisfactory type of protection tube.

alkali and 0.10 normal acid, but, with such a weak alkali, the reaction with sulphur proceeds very slowly, thus greatly increasing the time of heating.

It is not claimed that this method is sufficiently accurate for delicate chemical analyses. However, the method seems to be entirely satisfactory for the purpose for which it was developed. It has the advantage of requiring much less time and equipment than does the gravimetric method and should prove useful wherever approximate quantitative analyses of free sulphur are desired, provided the material to be analyzed does not contain impurities that will react with the sulphur or with sodium hydroxide.

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PHYTOPATHOLOGICAL NOTES

A New Species of Graphium Causing Lumber Stain.—A red-headed species of Graphium has been found frequently on freshly cut sapwood in forests and lumber yards. It has thus far occurred always associated with other blue-stain fungi, *Ceratostomella pilifera* (Fr.) Wint., *C. pluriannulata* Hedg., and *Graphium rigidum* (Pers.) Sacc.

This fungus has been collected in Ohio, Arkansas, Mississippi, and South Carolina. In forests it occurred on freshly felled *Populus deltoides* Marshall, *Quercus alba* L. and *Q. lyrata* Walter and in lumber yards on sap wood of *Liquidambar styraciflua* L. and on a Pinus log.

The synnemata of the fungus are noticeable because of the carmine[1] color of the droplets on the tips of the stalks. This carmine-colored mucous matrix, which holds the primary conidia, is soluble in water. It sometimes flows down the stalks and stains the surrounding surface of the wood making it look as if splashed with blood. The external red stain is transitory and of minor importance, compared to the blue stain caused by the vegetative hyphae growing in the wood cells.

To test the staining ability of this Graphium, sticks of freshly cut sap wood of sweet gum (*Liquidambar styraciflua*) were inoculated with a pure strain of the fungus, which had been isolated from sap gum. The wood was stained gray or grayish black when damp. The fungus fruited vigorously. Pine, *Pinus echinata* Mill. and *P. taeda* L., was inoculated with strains isolated from gum. The wood was stained a light gray. The fungus fruited readily. The stain it caused is not so dark as that caused by the common yard stain fungi, *Ceratostomella pilifera* and *Graphium rigidum*, and it is not so vigorous in culture. The writer published a preliminary description of this fungus in 1911.[2]

Graphium rubrum sp. nov.

DIAGNOSIS

Stipitibus fertilibus 780μ altis, 45μ crassis, fuscis nigrescentibus. Capitibus ex hyphis hyalinis compositis conidia prima acrogena gerentibus. Conidiis in carmine coloratos mucosos, globosos, 200μ crassos cadentibus, oblongis, hyalinis, 2×4μ. Mycelio septato, 1–4μ, nigrescente. Conidiis secundariis hyalinis, claviformibus ex conidiophoris erectis nascentibus, acrogenis elatis et capitulatis, forma diversis.

[1] Ridgway, Robert. Color Standards and Nomenclature. Washington, D. C. 1912.

[2] Über die Einwirkung des Säure und Alkaligehaltes des Närbodens auf das Wachstum der holzzersetzenden und holzverfärbenden Pilze, mit einer Erörterung über die systematischen Beziehungen zwischen Ceratostomella und Graphium. Naturwissenschaftliche Zeitschrift für Forst und Landwirtschaft **9**: 429–467. 1911.

Hab. in ligno sapido Quercus albae, Populi deltoidis, Quercus lyratae, Liquidambaris styracifluae et Pini sp. in America boreale.

Graphium rubrum sp. nov.

TECHNICAL DESCRIPTION

Synnemata dark brown or black, expanded at base, 480–2000 μ, average 780 μ, in height, and 9–86 μ, average 45 μ, in diameter (Fig. 1); head com-

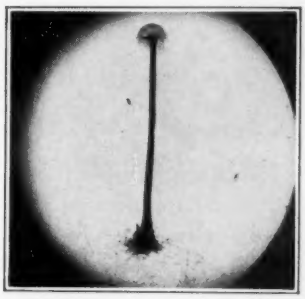

FIG. 1. Synnema of *Graphium rubrum* without the carmine mucous globule covering the head. × 75.

posed of hyaline branched hyphae bearing primary conidia terminally by abstriction; conidia drop into a carmine-colored mucous that forms a globule covering the surface of the head; globule 18–425 μ, average 200 μ, in diameter; young synnemata frequently grow from the heads of old ones; there is occasional proliferation of the synnemata. Primary conidia oblong, hyaline, average 2 × 4 μ; under favorable conditions conidia germinate producing mycelium; under unfavorable, they bud like yeast. Hyphae septate, 1–4 μ, in diameter; the young culture hyaline, changing with age to gray, slate, and slate black. Slow-growing mycelium forms dense, white, glistening mats of hyphae and sometimes sterile, clavariform fascicles. Secondary conidia club-shape, hyaline, develop first on hyphae, later on simple erect conidiophores, borne at the tips and collecting in clusters; vary in size and shape 3.2 × 8.5 μ to 2 × 14 μ, average 2 × 6.5 μ.—CAROLINE T. RUMBOLD, Division of Forest Pathology, Bureau of Plant Industry, in cooperation with Forest Products Laboratory, Forest Service, United States Department of Agriculture, Madison, Wisconsin.

A Simple Method of Obtaining Pythium Cultures Free from Bacteria.[1]—Bacterial contaminants, such as occur sometimes in newly isolated cultures of fungi, may be removed by acidifying the culture medium or by cutting

[1] Contribution No. 375 from the Division of Botany, Experimental Farms Branch, Department of Agriculture, Ottawa, Ontario, Canada.

out and transferring to a fresh medium uncontaminated portions of a culture growing on a solid medium. If, however, bacteria occur in a culture of Pythium, they are usually very persistent and frequently destroy the fungus. Because Pythium does not thrive on acidified media and because bacteria on neutral or alkaline media can multiply fast enough to keep pace with the growing tips of the hyphae, there is difficulty in obtaining uncontaminated Pythium cultures.

It is well known that fungi are able to grow within a solid medium as well as on the surface. The bacteria that contaminate fungal cultures, being usually obligate aerobes, are confined to the surface. If, therefore, the lateral spread of the bacterial colony on the surface of the medium is hindered or prevented, and the subsurface hyphae continue their growth, a pure culture of the fungus may be secured.

Brown[2] has made use of this knowledge in developing a method for the purification of fungal cultures. By inverting a portion of solid medium occupied by the contaminated culture over an unused part of the medium in the same Petri dish, he was able to obtain, within a few days, a pure culture of the fungus from the hyphae that had grown up through the inverted medium to its now upper surface.

Sometimes this method is not effective, particularly with some types of bacterial contaminants. Motile bacteria may escape through the film of liquid that separates the two layers of medium, particularly if the bacteria are of the rapidly multiplying, "Spreader" type; consequently, the culture may become recontaminated. Furthermore, if the medium be soft it is difficult to invert, and, if hard, cracking of the medium may occur over the inoculum and the bacteria may then spread through this avenue to the upper surface. To obviate these difficulties, another method, which has proved very satisfactory in culturing Pythium, has been developed. The same principle is involved. Instead, however, of inverting one layer of solid medium over another layer, the contaminated inoculum is covered with a sterile small glass disc. For this purpose a circular cover-glass, 20 mm. in diameter, has been found very satisfactory.

Melted potato-dextrose agar is poured into a sterile Petri dish. While the medium is yet soft, but cool enough so that the fungus is not killed, a small quantity of the contaminated Pythium culture is placed on the medium and immediately covered by the glass disc. The disc should be pressed down until it is in complete contact with the medium.

The glass disc prevents the lateral spread of the bacteria on the surface of the medium but does not hinder the growth of subsurface hyphae. If

[2] Brown, W. Two mycological methods. I. A simple method of freeing fungal cultures from bacteria. II. A method of isolating single strains of fungi by cutting out a hyphal tip. Ann. Bot. 38: 401–404. 1924.

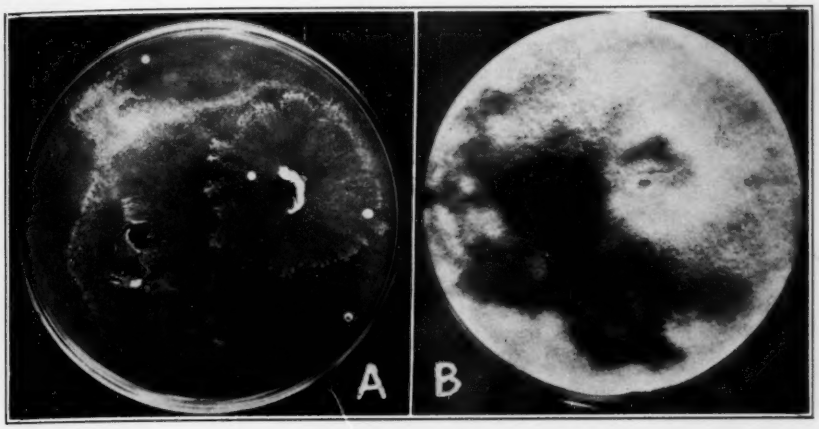

FIG. 1. Result of freeing a culture of Pythium from bacterial contaminants. A. Contaminated culture. B. Bacteria-free culture obtained by the glass-disc method.

the contaminated inoculum, thus transferred, has not been too adversely affected by the bacteria, the hyphae begin to emerge from beneath the disc within a few days. Transfers from the uncontaminated mycelium may then be made. The growth of the hyphae during the initial period may be observed by inverting a Petri dish and examining the culture under a microscope.

Figure 1 illustrates the efficacy of this method. The culture in Petri dish (A) is contaminated by bacteria. A portion of the culture has been transferred to Petri dish (B) and covered by the glass disc. After a few days the medium in the latter dish became overgrown by the fungus, particularly around the glass disc which is outlined by the matted hyphae. The whole culture in dish (B) outside of the disc is free from bacteria.—J. E. MACHACEK, Dominion Rust Research Laboratory, Winnipeg, Manitoba, Canada.

Cell Degeneration in Relation to Sieve-tube Differentiation in Curly-top Beets. A Preliminary Note.—Curly-top is primarily a disease of the phloem. This is the first tissue that shows degeneration and the only region that undergoes necrosis.[1] Moreover, a study of leaves of curly-top beets showed that the development of readily visible pathologic changes is definitely related to the differentiation of primary sieve tubes.[2] No cell degeneration could be observed in young leaves above the region of differentiation of primary sieve tubes, but, where these elements had matured,

[1] Esau, K. Pathologic changes in the anatomy of leaves of the sugar beet, *Beta vulgaris* L., affected by curly top. Phytopath. 23: 679–712. 1933.

[2] Esau, K. Ontogeny of the phloem in sugar beets affected by the curly-top disease. Amer. Jour. Bot. 1934. In print.

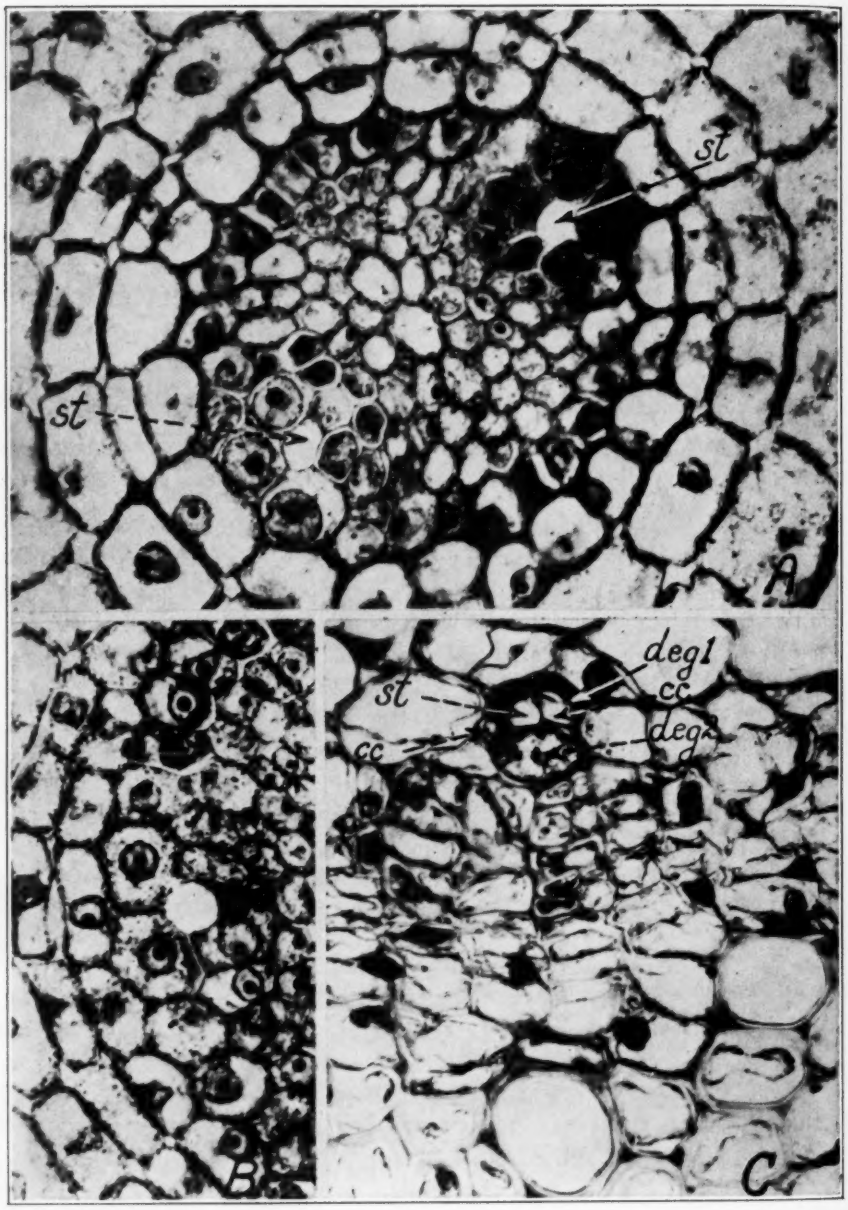

FIG. 1. A. Stele of a diseased root tip. B. Protophloem sieve tube with surrounding cells from a healthy root tip. C. Bundle from the third vascular ring of a diseased root. cc, companion cell; deg, degenerated cells; st, sieve tube. All ×797.

the cells surrounding them developed abnormalities characteristic of curly-top phloem. Recent studies on the root confirmed these observations.

In the central cylinder of the primary root the first cells to mature are two protophloem sieve tubes located in transverse sections on opposite sides of the stele. Figure 1, B, shows a portion of the stele of a healthy root tip, the protophloem sieve tube appearing as though devoid of contents. The cells to the right of the sieve tube are young phloem cells, whereas the two large cells to the left belong to the pericycle.

In the root tips of diseased beets unmistakable signs of degeneration characteristic of curly top may first be observed in cells lying adjacent to the protophloem sieve tubes. These cells develop inclusion bodies and show hypertrophic and necrotic changes. Figure 1, A, represents a transverse section of the stele of a diseased root tip. The 2 apparently normal protophloem sieve tubes (st) are conspicuous by scarcity of contents. Degeneration had not yet progressed very far in the neighborhood of the sieve tube to the left, but 2 cells of the pericycle and 1 of the phloem showed a pronounced hypertrophy of nuclei. To the right, the cells adjacent to the sieve tube showed darkly stained disintegrating protoplasts. In later stages of infection degeneration spreads to other cells in the phloem region, causing the development of a peculiar hyperplastic tissue.[2]

When secondary growth occurs in the beet, successive concentric rings of vascular tissue develop in the fleshy portion of the root. In diseased plants phloem degeneration spreads to the newly formed rings. But here also sieve tubes that are apparently normal develop before degeneration sets in. Figure 1, C, shows a transverse section of a bundle taken from the third and youngest vascular ring in a young beet root. In the upper part of the figure is a sieve tube (st) that is the first to develop in this bundle. It is flanked by companion cells (cc) on 2 sides. The darkly stained structure above the sieve tube (deg 1) is a collapsed parenchyma cell that had undergone degeneration and necrosis. The parenchyma cell below the sieve tube (deg 2) shows a protoplast in an early stage of disintegration. The tissue layers farther below are represented by phloem, cambium and xylem. The phloem shows abnormalities that will not be described here.

The sieve tube seems to play a definite rôle in the initiation of degenerative changes in curly-top-diseased beets. Perhaps it represents the channel through which the virus is translocated to the newly developing parts of the plant.—KATHERINE ESAU, University of California, Davis, California.

Powdery Mildew of Flax in Minnesota.—In September, 1933, the writer observed a powdery mildew on flax in the Coon Creek experimental peat plots near Anoka, Minnesota. The only varieties heavily infected were C. I. 669 and C. I. 743, although a few other varieties and hybrids were very lightly infected.

The mildew was found on the leaves, stems, and sepals. Perithecia were found only on the 2 heavily infected varieties. They were not numerous but often appeared in clusters on all of the plant parts attacked. The conidial stage was produced in the greenhouse 18 days after inoculation.

As far as the writer can determine, this is the first recorded appearance of flax mildew in North America, although it has been reported in Europe and Japan.[1, 2] Škorić[3] first described the conidial stage, giving it the name, *Oidium lini,* n. sp. Homma, in 1927, according to her later publication,[2] reported a flax mildew that produced perithecia. She identified it as *Erysiphe polygoni* DC. Later she described the conidial stage of another mildew on flax that she considered identical with *Oidium lini* Škorić but

TABLE 1.—*Species identification and dimensions of conidia of flax mildew as given by various investigators.*

Investigator	Species	Length		Width	
		Range in μ	Mean in μ	Range in μ	Mean in μ
Homma	E. polygoni	30.0–38.4		19.4–24.0	
Homma	O. lini	26.4–36.0		12.0–14.4	
Škorić	O. lini	26–41		12–15	
Salmon and Ware	E. polygoni (probably)	31–40	34	12–16	14
Writer	E. cichoracearum	23.1–33.5	29.4 ± 0.16	12–18	15.1 ± 0.09

distinctly different from *E. polygoni.* Badayeva[4] reported a powdery mildew, *E. cichoracearum* DC., as one of the most prevalent diseases on flax in Siberia.

The perithecia of the flax mildew collected in Minnesota were reddish brown, with numerous flexuous, darkened, simple appendages. The mean diameter of 50 perithecia was 112.5 ± 0.37 μ with a range of 84.7–142.7 μ. The perithecia usually contained 8 to 12 stalked asci. The mean width of

[1] Salmon, E. S., and W. M. Ware. The powdery mildew of flax. Gard. Chron. **82**: 34–35. 1927.

[2] Homma, Yasu. On the powdery mildew of flax. Bot. Mag. (Tokyo) **42**: 331–334. 1928.

[3] Škorić, Vladimir. Erysiphaceae Croatiae. Prilog fitopatološko-sistematskoj monografiji naših pepelnica. Glasnik za Šumske Pokuse. (Annales pro Experiments Foresticis) Zagreb. **1**: 52–118. 1926.

[4] Badayeva, P. K. O bolezniokh l'na v Sibiri. Morbi Plantarum, Leningrad, **19**: 192–199. 1930. (Abs.) Rev. Appl. Myc. **10**: 459. 1931.

Paper No. 1237 of the Journal Series of the Minnesota Agricultural Experiment Station.

100 asci was 27.9 ± 0.20 μ and the range 21.3–34.6μ. The asci contained predominantly 2 ascospores, rarely 1 or 3. The mean width of 100 ascospores was 11.6 ± 0.07μ, and the range was 9.4–14.6μ. The mean length was 21.4 ± 0.13μ with a range of 15.9–27.3μ. On the basis of these characters and measurements the mildew was identified as *Erysiphe cichoracearum* DC.

Measurements of the conidia obtained by the writer are compared in table 1 with the conidial measurements obtained by Homma, by Škorić, and by Salmon and Ware. The range in width of the conidia of the Minnesota mildew is outside that given by Homma for *Erysiphe polygoni* but agrees with that given by Škorić and Homma for *Oidium lini*, and by Salmon and Ware for the mildew that they described. The range in length is different from that for *E. polygoni* but very similar to that given by Škorić and Homma for *O. lini*. The range in length of conidia reported by Salmon and Ware is approximately the same as that given by Homma for *E. polygoni*. However, since the width of conidia of powdery mildews is considered more constant than the length, it seems more likely that the mildew described by Salmon and Ware is the same as that described in this paper. The conidial stage of the mildew found in Minnesota appears to be the same as *O. lini* described by Škorić and Homma. On the basis of conidial and perithecial characters the mildew is *E. cichoracearum*.—C. C. ALLISON, Minnesota Agricultural Experiment Station, St. Paul, Minnesota.

Olive Knot on Olea chrysophylla.—*Olea chrysophylla* Lam. has been distributed at different times by the United States Department of Agriculture. It is native to the highlands of East Africa and differs from *O. europea* L. in having leaves whose dorsal surface is yellowish or drab instead of light green (almost white).

Olea chrysophylla has been successfully inoculated with the olive-knot organism, *Bacterium savastanoi*. (Fig. 1.). The resulting knots, however, differ in appearance from those occurring on *O. europea*. They are smaller, less globose, and rougher or more irregular in form. In appearance they resemble more those produced by *Bact. savastanoi* on certain hosts[1] allied to the olive, such as Fraxinus, Forestieria, and Osmanthus. They also have a general resemblance to galls produced by *Bact. tumefaciens*, but *Bact. tumefaciens* is not known to make galls on *O. europea*, the source from which these original cultures were isolated.

The inoculations on the 2 hosts were made by using 2 different cultures of *Bacterium savastanoi*, and a period of 13 months elapsed between the 2 series of inoculations.

[1] Smith, C. O. Pathogenicity of the olive knot organism on hosts related to the olive. Phytopath. **12**: 271–278. 1922.

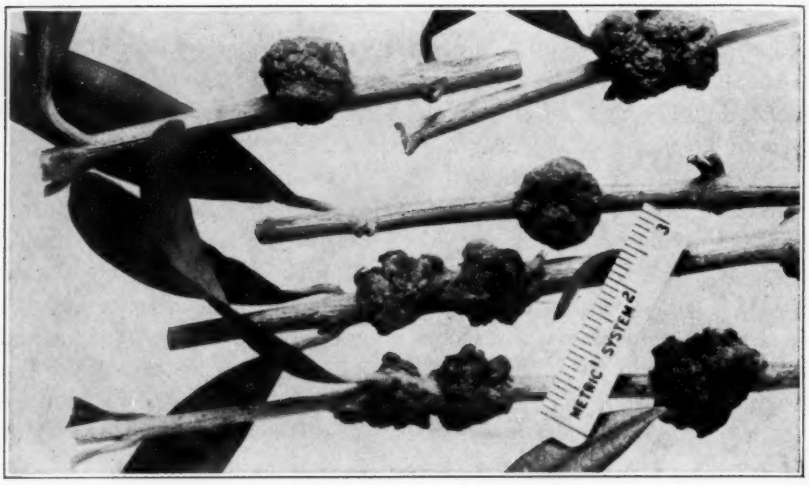

FIG. 1. Knots on *Olea chrysophylla* 20 months after inoculation marked by metric measure. Knots above on *Olea europea* after 7 months.

The knots on *Olea chrysophylla* were photographed 20 months and those of *O. europea* 7 months after inoculation. By that time these knots should have reached their final size and form and would show any difference in character of knot. The differences as given are believed to be due to a difference in growth responses between the causal organism and the individual hosts.

The causal organism was reisolated from the artificial knots on *Olea chrysophylla,* and reinoculations into *O. europea* and *O. chrysophylla* produced knots typical for each of the two hosts.—CLAYTON O. SMITH, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

Color in Relation to Virulence in Aplanobacter michiganense.—Since April, 1931, studies have been in progress on color variation in *Aplanobacter michiganense,* the cause of bacterial canker of tomatoes,[1,2] with the object of discovering the significance of these variations and the extent to which they may be amenable to experimental control.

Both the serial culture method of Hadley,[3] using beef bouillons adjusted

[1] Bryan, Mary K. An albino strain of *Aplanobacter michiganense.* (Abst.) Phytopath. 20: 141. 1930.

[2] Bryan, Mary K. Color variations in *Aplanobacter michiganense.* Phytopath. 21: 559. 1931.

[3] Hadley, P., E. Delves, and J. Klimek. The filtrable forms of bacteria: I. A filtrable stage in the life history of the Shiga dysentenry bacillus. Jour. Inf. Dis. 48: 1–159. 1931.

to pH 6.0, pH 7.0 and pH 8.0, and the technic described by Quirk[4] for the production of rough and smooth colonies, modified to suit the needs of this slow-growing organism, were employed.

Preliminary experiments with laboratory stock cultures of the *yellow*, *white* and *pink* forms showed the pink one to be most readily affected by environmental conditions. Both this earlier work and later experiments with a *single-cell* strain of the *pink* form indicate (1) that the pH of the media, the age of the culture, the amount of dilution to which it is subjected, and the age of the dilutions when plated all may be factors in controlling the form and color of colonies obtained; (2) that color changes bear a definite relation to the rough and smooth types or stages in the life of a culture and (3) that *color* as well as colony form is linked with degree of *virulence*.

The writers have repeatedly obtained experimentally the yellow and white forms from the pink one and have consistently found them both to be markedly more infectious than the parent culture, the pink form in many instances being nonvirulent.

Within any one color strain both rough and smooth colonies occur and, when working with the pink strain, a wide range of color and form has been found, including intermediates that combine the qualities of roughness and smoothness and appear to represent blends of "pink," yellow and white to form salmons, buffs, and oranges of different intensities.

An article treating of certain phases of the work is planned in the near future.—EDNA H. FAWCETT and MARY K. BRYAN, Bureau of Plant Industry, Washington, D. C.

The Host Specificity of Septoria petroselini and S. apii-graveolentis.—The question of specificity of two species of Septoria affecting celery and facts concerning their distinction from the parsley Septoria was reported by the author[1] in 1932. In that work neither of the celery forms, although they had both been called *Septoria petroselini*, were found to infect parsley, thus for purposes of clarifying nomenclature were concluded to be specific to celery. Aside from the desirability of testing *S. petroselini* on celery, a culture could not be obtained at that time.

Recently, cultures of *Septoria petroselini* and *S. apii-graveolentis* were sent to the writer by Dr. Johanna Westerdijk from the Central-Bureau voor Schimmelcultures of Baarn, The Netherlands. Two lots of twenty celery plants were inoculated respectively with the organisms and infection occurred uniformly on the plants inoculated with *S. apii-graveolentis*. The

[4] Quirk, Agnes J. Pure smooth and rough colony types at will. Science n. s. 74: 461. 1931.

[1] Phytopath. 22: 791–812. 1932.

same experiment was repeated using parsley plants and infection resulted only on the plants inoculated with *S. petroselini*. Thus it is entirely clear that these two organisms are specific to their respective hosts and the separation of the celery Septorias from *S. petroselini* further justified.—L. C. COCHRAN, Mich. Agr. Exp. Sta., East Lansing, Mich.

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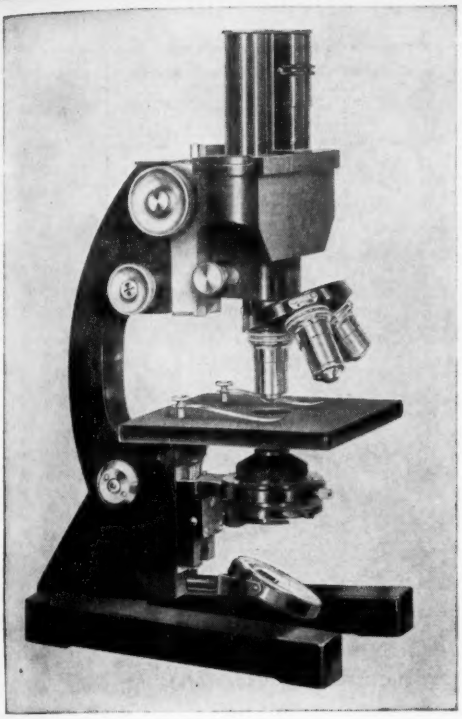

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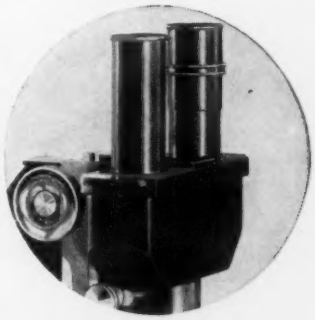

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